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FUSARIA INOCULATION EXPERIMENTS. RELATIONSHIP OF VARIOUS SPECIES OF FUSARIA TO WILT AND COLORADO DISEASE OF BANANA

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INTRODUCTION

During an investigation conducted on Fusaria in relation to banana plantations in Honduras, isolations were made from living and dead parts of diseased and non-diseased bananas; from living and dead parts of other plants growing in banana plantations and in the virgin forest to be planted to bananas; and from the virgin soil and soil in diseased and non-diseased banana plantations. Special isolations were made from banana plants affected with wilt and the Colorado disease to determine whether more than one type of *Fusarium* or any other organism was associated with these diseases. Other studies on banana wilt have shown that *Fusarium cubense* is definitely associated with the banana wilt plants and that generally no other *Fusarium* is found in typical cases of the disease.[2]

Only in two cases were other Fusaria found. One of these, an old infection, appeared to be a combination of banana wilt and Colorado disease. It was not a typical case of wilt. *Fusarium moniliforme* var. *subglutinans* Wr. and Rkg, was found in the vascular bundles of this plant. Another plant affected with banana wilt yielded *Fusarium lutulatum* Sherb. from its vascular bundles. In both of these cases the isolations were made from material that had been collected one and three days respectively before the isolations were made. This interval might well have given these doubtful fungi a chance to enter the diseased tissue from the cut surfaces. In any event, because of the extensive number of isolations made under controlled conditions from wilt diseased plants, it might well be stated from this standpoint that *Fusarium cubense* only is associated with typical cases of banana wilt. Examinations made of plants infected with the Colorado disease

[1] Pathologist, United Fruit Co.

[2] Brandes, E. W. Banana wilt. Phytopath. **9**: 339–389. 1919.

indicated that this trouble was due primarily to an abnormal cultural condition and not to fungi. Because, however, one fungus *Fusarium moniliforme* var. *subglutinans*, was commonly associated with the diseased plants and their environs, it was deemed advisable to use this organism for inoculation purposes to determine whether it was pathogenic and could produce the symptoms of Colorado disease.

FIG. 1. Inoculation experimental plot I. Concrete tanks built in the ground. Four and one-half feet square.

Soil isolations and those from banana, and other than banana plants, were made primarily to determine the distribution of *F. cubense* in diseased plantations and in virgin forests and soil. Isolations made from the soil in diseased and non-diseased plantations and virgin soil showed that there are a few closely related organisms that could be confused with *F. cubense*. These are *Fusarium oxysporum*, *F. oxysporum* var. *nicotianae*, *F. orthoceras*, and *F. orthoceras* var. *triseptatum*. Because of these closely

related forms, all of which have been found to be parasitic on plants in various parts of the world, it was found necessary to make inoculations to determine their pathogenicity and their relationship with *F. cubense* and with banana wilt.

A very careful morphological and physiological study of these and all other species collected was made in order to establish definitely their relationship. These studies culminated in the Fusarium conference held at Madison, Wisconsin.[3] Here a special study was conducted with Dr. H. W. Wollenweber on the entire collection of tropical Fusaria. Out of the collection of some 250 strains, 48 different species and varieties of Fusaria were described. These included species in practically all of the sections of the genus. Fourteen forms were described as new.[4] It might be stated here that these morphological studies of the organisms concerned corroborated very closely the results of inoculations made in the tropics. The morphological studies showed that a close relationship exists between *F. cubense* and species of the Oxysporum group. *F. oxysporum* var. *nicotianae* was found to be very closely related, although a slight difference was always present. These findings then leave one question open, and that is, whether a saprophytic form of *Fusarium* closely related to *F. cubense* can eventually, by a gradual process, develop the parasitic qualities necessary to produce disease in bananas. It is believed, however, that the following inoculation experiments, especially when correlated with isolation and morphological studies, show that there is a definite parasitic strain, *F. cubense*, that only can produce the typical banana wilt disease.

METHODS

The inoculation experiments, as planned, might well be divided into two parts: one, the preliminary experiments, carried out to determine whether the proposed method of procedure was correct; two, the main experiment.

PRELIMINARY EXPERIMENT

In the preliminary experiment, two wooden boxes and one concrete tank, all placed above the ground (Figs. 2 and 3), were used for the trials. Since the procedure was the same as that carried out in the main experiment, no detailed explanation will be given. It will suffice to say that cultures of *F. cubense* isolated from diseased plants (table 2) were used for inoculation. The results of this experiment are given in table 1.

The results shown in table 1 proved that the banana wilt disease could be produced in plants growing under the particular conditions devised for

[3] June, July, and part of August, 1924.

[4] Wollenweber, H. W., and O. A. Reinking. Aliquot Fusaria tropicalia nova vel revisa. Phytopath. **15**: 155–169. 1925.

TABLE 1.—*Preliminary inoculation experiments with F. cubense*

No.	Culture used	Planted and inoculated 1922	Type of soil	Cut down 1923	Wilt disease
Box 1	R 11. *F. cubense*	Dec. 20	Fine silt	July 13	X[a]
Box 2	R 3. *F. cubense*	do	do	do	—
Tank 1	R 2. *F. cubense*	Dec. 30	do	do	X

[a] X disease present; — disease absent.

the experiment (Fig. 6). The methods of soil sterilization, planting, and inoculation were found to be successful. These are discussed under the main experiment.

MAIN EXPERIMENT

Plan of experiment. The main experiment was divided into three parts, with the idea of providing a careful check upon the different methods used, and to produce conditions that were favorable to infection. The same organisms, therefore, were tested under two, and, in four cases, under three different conditions. The first experimental plot (Fig. 1) consisted of 24 concrete tanks built into the ground. Four of these tanks were 4½ feet wide, 9 feet long, and 4 feet deep. The other tanks were 4½ feet square and 4 feet deep. A fine wire mesh fence encircled the entire plot so as to exclude rodents and other animals.

The second experimental plot (Fig. 2) consisted of 16 wooden boxes, 2½ feet square and deep, with wooden bottoms. These boxes were raised above the ground by means of pieces of timber.

The third experimental plot (Fig. 3) consisted of four concrete tanks, 4 feet square and deep, inside measurement, placed on the ground. Cement bases were provided for these boxes.

These three experimental plots gave a variety of methods, with different degrees of control from outside sources of infection, and also different growth conditions, varying from a natural condition to one not so well adapted to the growth of bananas. The boxes and concrete tanks placed on the ground gave a condition that was less apt to be influenced by ouside infection, but at the same time did not present a natural condition for growth. The concrete tanks placed above the ground, however, were large enough to grow perfectly healthy, normal plants.

Soil. Because it was known that the soil has a relation to disease production, two types of soil were used for the experiments: one a fine silt, approximately neutral soil, and the other a light, sandy, acid soil. The disease apparently does not spread so rapidly in the former soil as in the latter. The latter soil gave a condition under which the organisms used

for inoculation would have the best possible opportunity to prove their pathogenicity. The disposal of these soils in the various experiments is given in tables 2, 3, and 4.

FIG. 2. One row of inoculation experimental plot II, using boxes of sterilized soil, above the ground, for planting. Banana wilt disease produced in Box No. 1 by inoculation with pure culture R 1, *F. cubense* isolated from vascular bundles in rhizome of wilt diseased banana plant. Note characteristic splitting of pseudostem and bending over of diseased leaves.

Sterilization of soil. The soil was sterilized by means of a special apparatus consisting of ¾-inch steam piping perforated with holes and so arranged that jets of steam from these perforations would reach all particles of soil. The apparatus was designed in the form of a large fork, with prongs of perforated tubing so designed that each tube was not over 6 inches from any portion of the soil in the entire tank. The forked apparatus was driven into the soil in the tanks and connected with a steam hose leading to a boiler. The top of the soil in the tanks was covered with a double layer of moistened, heavy burlap bags. For experimental Plot I, a steam boiler was used to provide the steam for sterilization. The soil in each tank was sterilized for two to three hours with live steam, derived

FIG. 3. Inoculation experimental plot III. Concrete tanks, with cement bases, above the ground. Four feet square inside.

at 110–120 pounds pressure in the boiler. The temperature maintained in the soil for this time was from 100° to 102° C. The soil in the wooden boxes of Plot II and the concrete tanks of Plot III were sterilized for two hours by live steam from a locomotive maintaining a pressure of 150 pounds; otherwise the operation was the same as in Plot I. The temperature of the soil in all parts of the boxes and tanks was 100–102° C. during the time of steaming. As soon as each tank or box of soil was sterilized, the sterilizing apparatus was removed, under sterile conditions, and a piece of heavy, sterilized, tarred paper, painted on both sides with a full strength solution

of carbolineum, was tightly fitted over the top of the soil. The soil in this way remained hot for two or three days after the time of sterilization. Tangle-foot was smeared over the top of each tank so as to prevent crawling insects from entering (Fig. 1). Each tank was planted with a banana plant, and the soil was inoculated as soon as it cooled off. Bacteriological and fungous tests made of the soil at the time of planting proved that the soil in every case was sterile except in Tank 4, Plot I, as shown in table 2.

It was found a little more difficult to sterilize the soil in the large $4\frac{1}{2} \times 9$ feet tanks, as only one half could be sterilized at one time with the apparatus at hand. Enough controls were maintained to prove the efficiency of the sterilization work.

"Bits" or rhizomes used for planting. The "bits" or portions of rhizomes used for planting were obtained from a district where the banana wilt disease was not found. These "bits" were carefully washed free from all dirt, trimmed up as much as possible with a sterilized machete so as to have clean-cut surfaces, and then sterilized for five minutes in a 2 to 1000 strength solution of mercuric chloride. They then were washed in sterile water and trimmed up again with a sterilized machete so as to remove as much as possible of the outer parts that may still have contained some of the poison. The "bits" were then immediately planted and the soil inoculated. Three "bits" were planted in the large concrete tanks in Plot I, and one "bit" in each of the other tanks and boxes.

Method of planting and inoculation. In planting, a square hole large enough to allow for the passage of the "bit" was cut into the center of the tar paper covering the soil in each tank. Under aseptic conditions a hole was dug in the soil, large enough for proper planting, and the "bit" inserted according to the general farm practice. Before covering with earth, the fungous culture used in each case was applied about the "bit" and in the soil. Cultures of the various fungi used for inoculation were made in pint fruit jars, using about one-half pint of a mixture of corn meal and soil upon which to grow the fungus. Other cultures were grown upon sterilized green bean pods in Erlenmeyer flasks, and in certain cases these were also applied. The cultures were from 8 to 15 days old at the time of application. They were applied by being poured over the "bits" and mixed with the soil about them. After the "bits" were entirely covered with soil, the flap of tarred paper was again dropped back over the soil, thereby completely covering the tank and preventing air-borne spores from entering the soil. This method gave the fungus used for inoculation an opportunity to spread and develop in a pure state throughout the soil about the "bit." As soon as suckers forced their way through the soil, the flaps of paper were thrown back to allow the plant to grow. All possible precautions were taken against contamination from outside sources. The plants thenceforward were allowed to care for themselves.

FIG. 4. Banana wilt disease produced by inoculation with pure culture R 17, *F. cubense* isolated from vascular bundles in rhizome of wilt diseased banana plant. Note characteristic wilting and drying up of leaves on young sucker in front. Tank No. 8.

CULTURES USED FOR INOCULATION

The cultures used for inoculation, as given below, were strains of the banana wilt organism (*F. cubense*); closely related organisms, isolated from the soil and plant parts, more or less difficult to distinguish from the wilt producing organism (*F. oxysporum* var. *nicotianae*, *F. orthoceras*, and *F. orthoceras* var. *triseptatum*); two organisms commonly associated with banana plants and frequently found about Colorado diseased plants (*F. moniliforme* var. *subglutinans*, and *F. moniliforme* var. *erumpens*); and two organisms, one commonly found on decaying banana trash (*F. anthophilum*) and the other in the soil (*Hypomyces ipomoeae*).

LIST OF CULTURES USED FOR INOCULATION

R 1. *Fusarium cubense.*

Isolated from vascular bundles in rhizome of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

R 3. *F. cubense.*

Isolated from vascular bundles in pseudostem of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

R 15. *F. cubense.*

Isolated from exterior of cut surface of pseudostem of wilt diseased Gros Michel banana (*Musa sapientum*). Truxillo, Honduras. Isolated Feb. 21, 1923.

R 17. *F. cubense.*

Isolated from vascular bundles in rhizome of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated April 15, 1923.

R 52. *F. cubense.*

Isolated from dead floral parts at tip of non-diseased banana fruit (*Musa sapientum*). Tela, Honduras. Isolated Jan. 12, 1923.

R 119. *F. oxysporum* var. *nicotianae.*

Isolation. Soil 1. Top soil under diseased bananas. Tela, Honduras. Isolated Nov. 17, 1922.

R 139. *F. oxysporum* var. *nicotianae.*

Isolation. Soil 35. Top soil under healthy bananas. Tela, Honduras. Isolated Jan. 15, 1923.

R 142. *F. oxysporum* var. *nicotianae.*

Isolation. Soil 38. Top soil under healthy bananas. Tela, Honduras. Isolated Jan. 15, 1923.

R 132. *F. orthoceras.*

Isolation. Soil 31. Top soil under healthy bananas. Tela, Honduras. Isolated Jan. 12. 1923.

R 45. *F. orthoceras* var. *triseptatum.*

Isolated from decaying old bunch of bananas (*Musa sapientum*) on ground. Tela, Honduras. Isolated Jan. 16, 1923.

R 182. *F. orthoceras* var. *triseptatum.*

Isolation. Soil 103. Top soil under diseased banana. Truxillo, Honduras. Feb. 23, 1923.

R 32. *F. moniliforme* var. *subglutinans.*

Isolated from decaying leaves of Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 17, 1922.

R 40. *F. moniliforme* var. *subglutinans.*

Isolated from vascular bundles in pseudostem of combination Colorado and wilt diseased banana plant (*Musa sapientum*). Isolation made Feb. 9, 1923, one day after cutting plant. Tela, Honduras.

R 58. *F. moniliforme* var. *subglutinans.*

Isolated from vascular bundles of pseudostem of Colorado diseased banana plant (*Musa sapientum*). Isolation made Feb. 9, 1923, one day after cutting plant. Tela, Honduras.

R 60. *F. moniliforme* var. *subglutinans.*

Isolated from vascular bundles of pseudostem of Colorado diseased banana plant (*Musa sapientum*). Isolation made Feb. 9, 1923, one day after cutting plant. Tela, Honduras.

R 62. *F. moniliforme* var. *crumpens.*

Isolated from vascular bundles of pseudostem of Colorado diseased banana plant (*Musa sapientum*). Isolation made Mar. 10, 1923, one day after collection. Tela, Honduras.

R 37. *F. anthophilum.*

Isolated from decaying banana trash (*Musa sapientum*). Tela, Honduras. Isolated Nov. 9, 1922.

R 184. *Hypomyces ipomoeae.*

Isolation. Soil 107. Top soil under healthy bananas. Truxillo, Honduras. Isolated Feb. 23, 1923.

PRELIMINARY INOCULATION EXPERIMENTS

R 2. *F. cubense.*

Isolated from vascular bundles in the rhizome of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

R 3. *F. cubense.*

Isolated from vascular bundles in pseudostem of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

R 11. *F. cubense.*

Isolated from vascular bundles in pseudostem of wilt diseased Gros Michel banana (*Musa sapientum*). Tela, Honduras. Isolated Nov. 16, 1922.

F. cubense isolated from wilt diseased plants was used primarily as a check on the other species. Brandes[5] has clearly shown that this organism is the cause of banana wilt. A very slight morphological and physiological difference was shown in some of these wilt-producing strains when not growing under normal conditions. Culture R 1 showed a tendency to run to mycelium and rarely produced spore bodies (sporodochia or pionnotes). *F. oxysporum* var. *nicotianae* isolated from the soil is very closely related to *F. cubense*, but does not produce the characteristic benzolic odor on rice

[5] Brandes, E. W. *Ibid.*

and has slightly larger spores and always a more abundant sclerotial formation. It is prevalent in the soil of diseased and non-diseased plantations. This resemblance made it necessary to test the pathogenicity of the fungus and in this way more clearly distinguish it, if possible, from *F. cubense*. *F. orthoceras* and *F. orthoceras* var. *triseptatum* are also commonly found in the soil and on decaying banana trash, and in some cases might be confused with a degenerate strain of *F. cubense*. Their relationship to *F. cubense*, therefore, also had to be tested out by the inoculation method.

FIG. 5. Banana wilt disease produced by inoculation with pure culture R 52, *F. cubense*, isolated from dead floral parts at tip of non-diseased banana fruit. Note splitting of pseudostem and characteristic bending over of diseased leaves. Box No. 9.

Another organism, *F. lutulatum*, is also closely related to *F. cubense*. This organism was not isolated until after the inoculation experiments were well under way. If future inoculation studies are undertaken, this species should be carefully tested. *F. moniliforme* var. *subglutinans* and *F. moniliforme* var. *erumpens* are distinctly different from *F. cubense* and could never be confused with the latter. They are commonly associated with banana culture and have been isolated from the vascular tissues of old Colorado diseased plants. Because of this relationship, these organisms were selected for inoculation purposes. The last two organisms used show no close relationship with *F. cubense*. *F. anthophilum* is commonly found on decaying banana trash, and the Fusarial stage of *Hypomyces ipomoeae* is quite common in the soil of infested and non-infested plantations. These species were used to determine their pathogenicity, and also to act as a check on the entire group.

INOCULATION EXPERIMENT. PLOT I.

Table 2 gives the details of the inoculation experiment (plot I) carried out in concrete tanks built into the ground (Fig. 1). The method of sterilization, planting, and inoculation followed were the same as discussed under methods.

All plantings and inoculations were made the latter part of June and the first part of July, 1923, as indicated in table 2. Final results in most cases were not obtained until April 26, 1924, some 10 months from the time of inoculation. At this time, fruits, just below the English grade for maturity, were produced in tanks 2, 11, 12, 13, 15, 16, 17, and 22. The flower had "shot" in tanks 5, 18, and 21. All other plants either produced no flower or fruit or were diseased. The production of fruit and the healthy appearance of the plants showed that they were growing under good conditions for fruit production.

All of the cultures were of *Fusarium cubense*, and all except one (table 1) were obtained from banana wilt disease plants. One culture, R 52, was isolated from the dead floral parts at the tip of a banana finger of a bunch piled in a car at a fruit spur. Whether the organism was present in the car or developed in the field of course could not be definitely determined. It, however, might be safe to assume, because of this evidence, that *F. cubense* may be present to a certain extent along the railroad right-of-way through a non-diseased district. From these inoculations, positive cases of infection were obtained in tanks 1, 3, 6, 8, and 23. No disease was produced in tank 7 inoculated with culture R 3, *F. cubense*. This same culture produced disease in tank 1. The reason for using culture number R 3 in duplicate was due to the fact that, while it was a typical culture of *F. cubense* from a physiological and morphological standpoint, it did not

TABLE 2.—*Results of inoculating with Fusarium banana plants growing in concrete tanks sunk into the ground*[f]

Tank No.	Culture used	Planted and inoculated	Type of soil	Cut down	Wilt disease
		1923		1924	
1	R 3. *F. cubense*	June 16[a]	Light Sandy	Mar. 29	X[e]
2	R 119. *F. oxysporum* var. *nicotianae*	do[a]	do	do	—
3	R 15. *F. cubense*	do[a]	do	Oct. 8 (1923) and Mar. 13	X
4	R 58. *F. moniliforme* var. *subglutinans*	do[a]	do	Mar. 13	X[b]
5	Control, sterilized soil	do[c]	do	Apr. 26	—
6	R 1. *F. cubense*	do[a]	do	Mar. 13	X
7	R 3. *F. cubense*	do[a]	Fine Silt	Apr. 26	—
8	R 17. *F. cubense*	do[a]	do	Mar. 13	X
9	R 37. *F. anthophilum*	June 19[a]	do	Apr. 26	—
10	Control, sterilized soil	do[c]	do	do	—
11	R 132. *F. orthoceras*	July 5	Light Sandy	do	—
12	R 184. *Hypomyces ipomoeae*	do	do	do	—
13	R 62. *F. moniliforme* var. *crumpens*	do	do	Apr. 12	—
14	Control, unsterilized soil	do[d]	do	Mar. 13	X
15	R 139. *F. oxysporum* var. *nicotianae*	do	do	Apr. 26	—
16	R 60. *F. moniliforme* var *subglutinans*	do	do	do	—
17	Control, sterilized soil	do[c]	do	do	—
18	R 182. *F. orthoceras* var. *triseptatum*	do	do	do	—
19	R 40. *F. moniliforme* var. *subglutinans*	do	do	do	—
20	R 142. *F. oxysporum* var. *nicotianae*	do	do	do	—
21	R 45. *F. orthoceras* var *triseptatum*	do[a]	do	do	—
22	R 32. *F. moniliforme* var. *subglutinans*	do	do	Apr. 12	—
23	R 52. *F. cubense*	do	do	Mar. 13	X
24	Control, sterilized soil	do[a]	do	do	—

[a] Replanted and reinoculated Aug. 10, 1923.

[b] Soil not thoroughly sterilized as shown by test at time of inoculation.

[c] Not inoculated; "Bit" planted in sterilized soil.

[d] Not inoculated; "Bit" planted in unsterilized, light sandy infected soil.

[e] X = disease present; — = disease absent.

[f] Tanks 1 to 4 inclusive, 6 and 7, 11 to 13 inclusive, 15 and 16, and 18 to 23 inclusive were inoculated with cultures growing on a mixture of cornmeal and soil and on green bean pods. Tanks 8 and 9 were inoculated with cultures growing on a mixture of cornmeal and soil. Controls, in which only sterilized bits were planted in sterilized soil and no fungous culture used, were scattered throughout the plot as represented in tanks 5, 10, 17, and 24. The tanks provided suitable checks upon the remaining inoculated ones. They were also checks to show whether or not the sterilization of the soil had been complete and whether there was any possible opportunity of infection from air-borne spores, insect-carried organisms or any other outside source. Tank 14 contained infested light sandy soil that had not been sterilized. This tank acted as a check on the sterilization of the infected soil and was used also to prove whether or not the organism causing the wilt disease was transmitted by the soil.

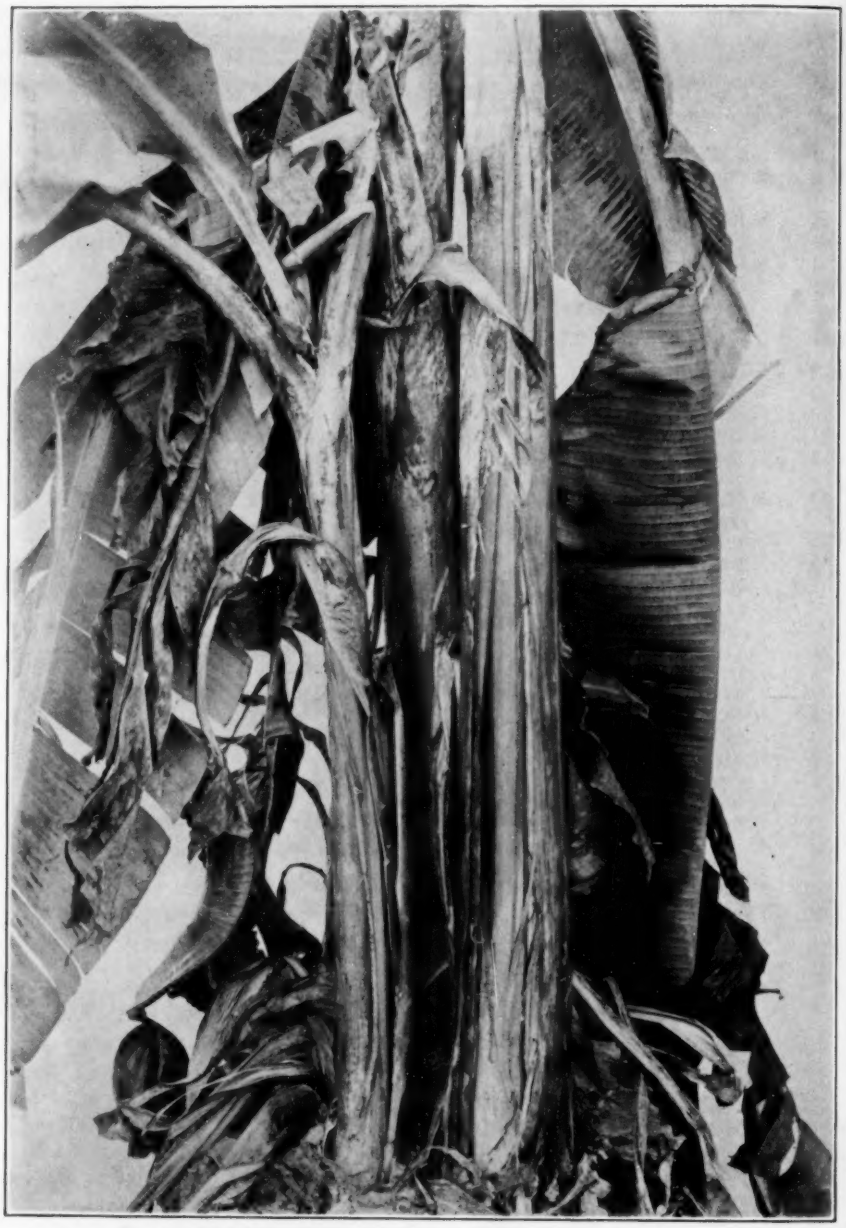

FIG. 6. Banana wilt disease produced by inoculation with pure culture R 2, *F. cubense*, isolated from the vascular bundles in the rhizome of a diseased banana plant. Note characteristic splitting of pseudostem. Preliminary inoculation experiment. Tank No. 1.

produce disease in the preliminary experiment as shown in table 1. Disease was also produced in tank 14, the control in which sterilized, infected, light sandy soil was used. This fact shows that the wilt organism occurs in the soil, and may be disseminated in soil. Disease was produced in one other case, in tank 4, inoculated with culture number R 58, *F. moniliforme* var. *subglutinans*. The disease was a typical case of banana wilt. Tests of the soil in this tank made at the time of inoculation showed the presence of Fusaria, indicating that the soil had not been thoroughly sterilized. The organism reisolated from the diseased plant was a pure culture of *F. cubense* and had no relation to *F. moniliforme* var. *subglutinans* used for inoculation. No significance, other than incomplete sterilization of soil, can be attached to this case of disease production.

The time of appearance of the disease after inoculation varied in the different tanks from two to seven and one-half months or longer. For example tank 1 was replanted and reinoculated on August 10, 1923. Disease was not recorded until March 29, 1924, just 7 months and 19 days after inoculation. Tank 3 was replanted and reinoculated on the same date. Disease appeared in one sucker on October 8, 1923, or two months after the time of inoculation. The disease did not appear in its virulent form until January 8, 1924, five months after inoculation.

The plants in all of the controls of sterilized and uninoculated soil, as represented by tanks 5, 10, 17, and 24, remained perfectly healthy. Tank 17 developed a bunch of fruit just below the English grade of maturity at the time of the close of the experiment on April 26, 1924.

No noticeable disease was produced by the inoculation of "bits" and soil with any of the other fungi used, including *F. oxysporum* var. *nicotianae*, *F. orthoceras*, *F. orthoceras* var. *triseptatum*, *F. moniliforme* var. *subglutinans*, *F. moniliforme* var. *erumpens*, *F. anthophilum*, and *Hypomyces ipomoeae*. In so far as these experiments are concerned, it was definitely proved that none of these cultures could produce disease either of the typical banana wilt type, or of the Colorado type. All the plants in these tanks were perfectly healthy and showed no evidence of root injury. It would not be safe to conclude that the first five of these fungi have no relation whatever to banana cultures. The possibility of a root infection being caused by some of this group has not been fully settled. The above experiments, however, are conclusive in regard to the direct role of these organisms in respect to the banana wilt and Colorado diseases.

In each case of disease production the original organism used for inoculation (*F. cubense*) was recovered in reisolations. The banana wilt organism was also definitely shown to be present with sporodochial production at the bend, on the exterior surface of diseased, bent-over leaves. Typical pure cultures of *F. cubense* were isolated from leaves of diseased

plants in tanks 3, 8, and 23. Positive isolations were also made from the leaves of the diseased plant in Box 9, Plot II. These results corroborate those obtained earlier by Brandes,[6] and show that the pathogene can be disseminated throughout the plantation by wind.

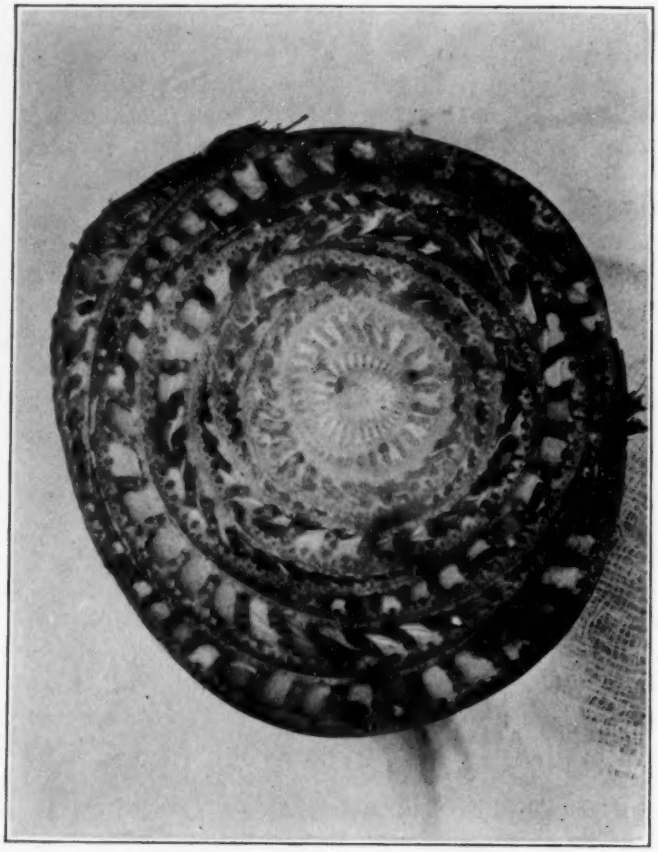

FIG. 7. Cross section of pseudostem of banana wilt diseased Gros Michel banana. Note discolored fibrovascular bundles more prevalent towards the exterior.

INOCULATION EXPERIMENT. PLOT II.

In order to have a check on the experiment carried out in Plot I, experimental Plot II was prepared with wooden boxes placed on the ground (Fig. 2). The purpose of the experiment was to provide a more careful control against possible outside sources of infection by raising the boxes off the ground, and also to make it possible to check up further the results obtained

[6] Brandes, E. W. *Ibid.*

by the cultures in Plot I. The details of the experiment are given in table 4. The arrangement, in so far as control and inoculation boxes are concerned, is similar to that discussed under Plot I.

TABLE 3.—*Results of inoculating with Fusarium banana plants growing in wooden boxes above ground*

Box No.	Culture used	Planted and inoculated	Type of soil	Cut down	Wilt disease
		1923		1924	
1	R 1. *F. cubense*	July 24	Fine Silt	Mar. 13	X
2	R 142. *F. oxysporum* var. *nicotianae*	do	do	Apr. 26	—
3	R 45. *F. orthoceras* var. *triseptatum*	July 18	do	do	—
4	R 139. *F. oxysporum* var. *nicotianae*	do	do	do	—
5	Control	do[a]	Light Sandy	do	—
6	R 60. *F. moniliforme* var. *subglutinans*	Aug. 10 July 18	do	do	—
7	R 182. *F. orthoceras* var. *triseptatum*	July 13	do	do	—
8	R 40. *F. moniliforme* var. *subglutinans*	do	do	do	
9	R 52. *F. cubense*	July 12	do	Mar. 13	X
10	R 45. *F. orthoceras* var. *triseptatum*	do	do	Apr. 26	—
11	R 15. *F. cubense*	do	do	do	—
12	Control	do[a]	do	do	—
13	R 3. *F. cubense*	July 6	do	do	—
14	R 119. *F. oxysporum* var. *nicotianae*	do	do	do	—
15	R 132. *F. orthoceras*	do	do	do	—
16	R 139. *F. oxysporum* var. *nicotianae*	do	do	do	—

[a] Not inoculated; "Bit" planted in sterilized soil.

As shown in table 3, the same cultures and soil used in Plot I were used in Plot II. The plantings and inoculations were made later, between July 6 and 18, 1923. The experiment was concluded April 26, 1924, or some 9 to 10 months later. Four different cultures of *F. cubense* were used, and, in two cases, in Boxes 1 and 9, typical wilt was produced. The interesting fact to note here is that the disease in these cases appeared approximately in the same time as it did when corresponding cultures were used for inoculation in Plot I, thereby checking up nicely on the pathogenicity of the cultures in question. Culture R 3 and R 15 failed to produce disease, although both were typical wilt-producing organisms and both had produced disease in Plot I. Box number 1 was inoculated with culture R 1 on July

24, 1923. On November 12, 1923, just 3 months and 19 days later, a decided splitting of the stem was observed. This splitting, and other symptoms of the disease, gradually became more pronounced (Plate 2). Box 9 was inoculated with culture R 52 on July 12, 1923. The first signs of the

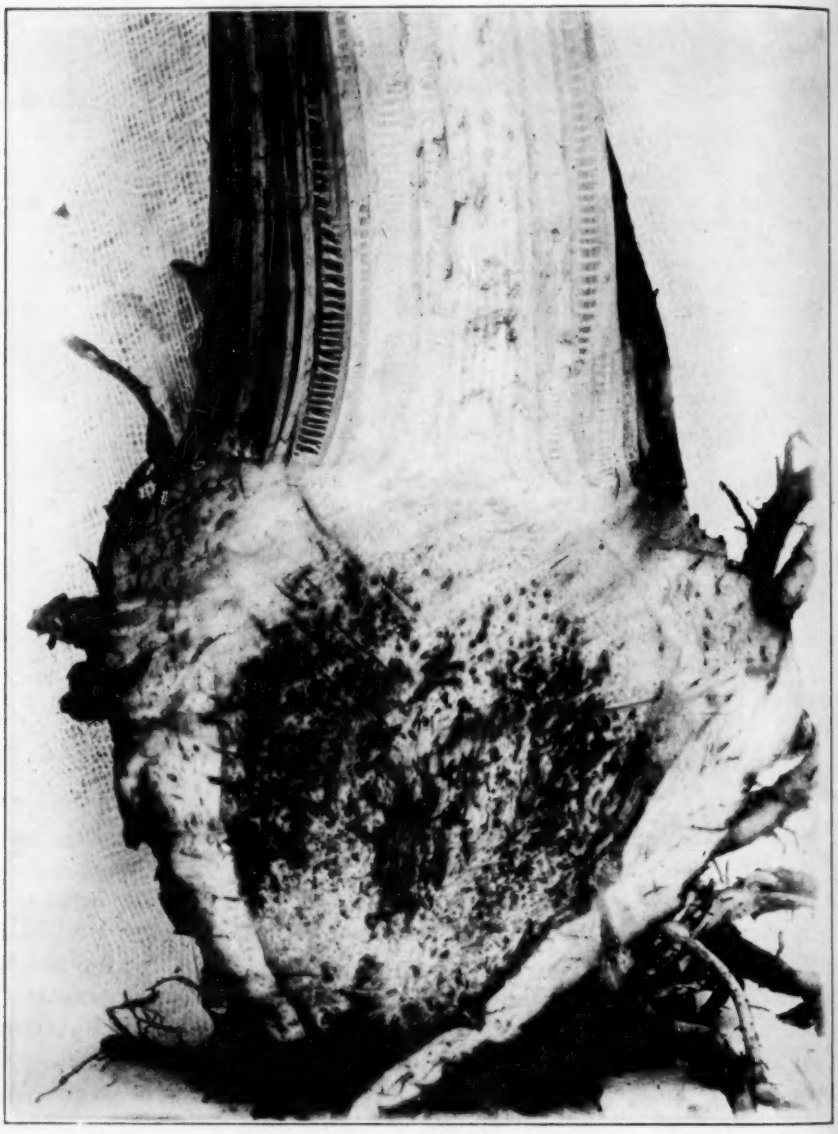

FIG. 8. Longitudinal section of banana wilt diseased Gros Michel banana. Note discolored fibrovascular bundles in rhizome and running into pseudostem.

typical disease were noted on December 5, 1923, not quite 6 months from the time of inoculation (Fig. 5). In no case was disease of any description produced in the other boxes inoculated with cultures coresponding to those used in Plot I, thereby verifying the results in the latter plot. The controls in every case remained perfectly healthy. Reisolations of the typical wilt organism similar to that used for inoculation were made from the two cases in which disease was produced.

INOCULATION EXPERIMENT. PLOT III

Experimental Plot III (Fig. 3) was a still further check on four cultures used in the main experiment, Plot I. The concrete tanks used in this experiment, being placed on the ground, were less subject to outside sources of infection. In the four tanks, representatives of the same cultures used in Plots I and II were used for inoculation, as shown in table 4. Inoculations were made on July 26, 1923, and the experiment was terminated on April 26, 1924.

TABLE 4.—*Results of inoculating with Fusarium banana plants growing in concrete tanks above ground*

Tank No.	Culture used	Planted and inoculated	Type of soil	Cut down	Wilt disease
		1923		1924	
1	R 119. *F. oxysporum* var. *nicotianae*	July 26	Fine Silt	Apr. 26	—
2	R 132. *F. orthoceras*	do	Light Sandy	do	—
3	R 139. *F. oxysporum* var. *nicotianae*	do	do	do	—
4	R 3. *F. cubense*	do[a]	do	do	—

[a] Reinoculated Nov. 1, 1923.

As indicated in table 4, only one culture of *F. cubense* (R. 3) was used for inoculation. No positive results were obtained with this culture. The failure of R 3 to produce disease in a number of the inoculations in Plots I, II, and III indicates that it is not extremely pathogenic under all conditions, although disease was produced by it in one instance (Plot I). The other cultures, R 119, *F. oxysporum* var. *nicotianae;* R 139, *F. oxysporum* var *nicotianae;* and R 132, *F. orthoceras,* again failed to produce disease of any kind, thereby verifying the results obtained in Plots I and II. Tank 3 at the close of the experiment had a bunch of fruit somewhat below the English grade in maturity. All of the plants in the experiment were perfectly healthy and normal.

DISCUSSION OF RESULTS

The results obtained in these experiments show conclusively that, of the organisms tested, only one distinct species, *F. cubense*, can produce the banana wilt disease. Isolation studies have shown that this one species only was found in all typical cases of banana wilt. Rarely have other Fusaria been found in the vascular bundles of diseased plants; and in these exceptional cases they have been isolated from plants with a complication of diseases, or after the portion of the plant from which the isolation was made had stood for a few days. It appears safe to say that *F. cubense* only can produce the typical banana wilt disease (Figs. 7 and 8), and that this organism only is found in young, typical cases of infection.

The role of other closely related species of Fusaria, especially *F. oxysporum* var. *nicotianae*, *F. orthoceras*, and *F. orthoceras* var. *triseptatum*, in relation to banana wilt appears to be negligible, as none of these were able to produce the disease. All three of the above organisms easily may be confused with *F. cubense*, especially when the latter is not in its normal state of development. This may also be true in regard to *F. oxysporum* and *F. lutulatum*, two species not tested from a pathogenic standpoint. When, however, *F. cubense* is in its typical and normal growth condition, the differences between it and the closely related forms are apparent. A detailed account of these differences will be given in a report on Fusaria in relation to banana plantations. In certain cases it still may be necessary to carry out inoculation experiments to distinguish definitely between a possible non-parasitic form of *F. cubense* and the parasitic species. Still further studies with these species might well be undertaken in the future to determine whether any root troubles can be produced by them.

While no culture of *F. cubense* isolated from the soil was used for inoculation purposes, it can safely be stated that this organism is present in the soil. This was clearly shown in the isolation, morphological, physiological, and inoculation studies. In the inoculation experiments, typical banana wilt disease was produced by planting a sterilized "bit" in a tank of unsterilized soil infested with *F. cubense*. *F. cubense* was reisolated from the diseased plant which showed the typical banana wilt symptoms. All precautions observed in the other tests were carried out in this tank experiment except that the soil was not sterilized. This test in itself shows conclusively that the causal organism is present in the soil and that it can be carried about by transporting infested soil.

The fact that the inoculation, morphological, and physiological studies have shown that a difference exists between *F. cubense* and the closely related forms found in and about banana plantations has a direct bearing on the question of the presence of *F. cubense* in virgin soil or upon dead and living vegetation found on, or growing in, virgin soil. These studies,

correlated with those made on the cultures isolated from several sources in and about banana plantations, including virgin soil and plants found thereon, makes it safe to say that the parasitic form of *F. cubense* is not present in virgin soil or upon dead or living plant material on lands that are virgin in so far as the banana plant is concerned. The organism apparently is closely confined to the banana plant and the soil in infected banana plantations. Once it becomes established in the soil, it retains its virulence for many years. There of course remains a possibility that a wild saprophytic form of *F. cubense* may be present in virgin soils and may gradually adapt itself to the banana plant and eventually assume a distinct parasitic nature. It is generally believed that this process of change requires many years. From a practical standpoint, however, it is safe to conclude that the distinctly parasitic form of *F. cubense* is definitely associated with the banana plant and infested banana plantations, and that the control measures will have to be based upon this conclusion.

The role of *F. moniliforme* var. *subglutinans* and *F. moniliforme* var. *erumpens*, the first of which is commonly found associated with living and decaying banana plants, has been in part rather definitely decided. *F. moniliforme* var. *subglutinans*, especially has been found to be associated with Colorado diseased plants. In order to test its pathogenicity by invading the plant through the underground parts, the comparatively large number of inoculations with this species were made. According to the results of these experiments, the organism, when inoculated into the soil and placed around the "bits" planted in the soil, has no definite parasitic nature. No disease was produced under the condition of the experiment. The test shows rather conclusively that the organisms in question have no direct relation to the Colorado disease, which may therefore be regarded as a disease produced by abnormal and poor cultural conditions. The organisms are extremely interesting because of their prevalence in and about banana plantations and might well bear watching.

The two other organisms tested, *F. anthophilum* and the Fusarial stage of *Hypomyces ipomoeae*, were definitely proved to be distinctly saprophytic in so far as the banana is concerned. The first species is very common on decaying banana trash, and the second is found on plants and in the soil of banana plantations and the forest. These organisms are not closely related to *F. cubense*.

SUMMARY

Only one distinct organism, *F. cubense*, is directly associated with typical cases of banana wilt and can produce the typical banana wilt disease.

Closely related species of Fusaria, especially *F. oxysporum* var. *nicotianae*, *F. orthoceras*, and *F. orthoceras* var. *triseptatum*, all of which are

found in diseased banana plantations, have no direct relation to banana wilt. The relation of the last two organisms to root troubles might well be studied further.

Fusarium cubense can be distinguished, in practically all cases, from closely related organisms. In certain doubtful cases, inoculation experiments will have to be resorted to in order to distinguish between a possible saprophytic form of *F. cubense* and the parasitic form.

Fusarium cubense is present in the soil, and from this source enters the plant and produces disease.

The inoculation studies correlated with the isolation, morphological, and physiological studies verify the statement made before, that *F. cubense* is not present in virgin soil in the parasitic form, which is closely associated with banana plants and the soil in infected banana plantations.

There is a possibility that a wild, saprophytic form of *F. cubense* is present in virgin soil and may gradually adapt itself to the banana plant and eventually assume a distinct parasitic nature. It is generally assumed that this process of change requires many years.

From a practical standpoint it is evident that the control work must be based upon the finding that the distinctly parasitic form of *F. cubense* is definitely associated with the banana plant and infested soil in banana plantations.

Fusarium moniliforme var. *subglutinans* and *F. moniliforme* var. *erumpens*, both quite generally found associated with living and decaying banana plants and often with the Colorado disease, have no direct relation to the Colorado disease. Because of the prevalence of these organisms in banana plantations, they might well bear watching.

Fusarium anthophilum, commonly associated with decaying banana trash, and the Fusarial stage of *Hypomyces ipomoeae*, found in soil and on dead plants in banana plantations and forests, have no direct relation to banana diseases.

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THE EFFECTIVENESS OF VARIOUS FUNGICIDES IN CONTROLLING THE COVERED SMUTS OF SMALL GRAINS

RESULTS OF THE COOPERATIVE CEREAL SEED TREATMENT PROJECT OF THE CROP PROTECTION INSTITUTE

COMPILED BY

E. B. LAMBERT, H. A. RODENHISER, H. H. FLOR[1]

INTRODUCTION

Until recently formaldehyde and copper sulphate were the standard fungicides in the United States for controlling the covered smuts of cereals. However, when these fungicides are applied to wheat or hulless oats, they sometimes cause more loss through seed injury than they prevent by controlling smut. For this reason several modifications of the treatments have been devised in order to eliminate seed injury. Some of them are efficacious, but they usually make the treatment more complicated and cumbersome.

The attention of most investigators in this field was still directed to the modification of old treatments when Darnell-Smith (1), in 1917, devised the copper carbonate dust treatment for bunt of wheat. The announcement of this discovery soon diverted the attention of investigators in the United States from the modification of old methods to the thorough testing of the copper carbonate treatment and to the devising of new treatments. Preliminary tests with copper carbonate (5) were so satisfactory that the American Phytopathological Society in 1922 recommended extensive cooperative tests with this material. It was pointed out that the quickest method of determining the practical advantages and limitations of the dust treatment would be through the united efforts of many workers in widely separated regions, in order to determine its utility under many different soil and climatic conditions. The Advisory Board suggested that the Crop Protection Institute handle the project. At the Rochester meeting of the Institute, Dr. C. R. Orton and Dr. E. C. Stakman were appointed a committee to organize the project and secure cooperators.

The objects of the project, as outlined by the committee, were to determine the effect of chemical dusts on the amount of smut produced, and on

[1] The writers assisted Dr. C. R. Orton and Dr. E. C. Stakman, the committee on cereal seed treatments of the Crop Protection Institute, in drawing up the project. They also handled the details of supplying the cooperators with the necessary materials, and compiled the annual reports. They wish to express their appreciation to Dr. E. C. Stakman for his helpful suggestions and criticisms in the preparation of this manuscript.

the germination and yield of wheat, oats, and barley, and to compare the general effectiveness of the dusts with that of copper sulphate and of formaldehyde. In the spring of 1922 the project statement was distributed to all members of the Institute and to those pathologists who the committee thought would be interested in cooperating. Investigators in eleven states in the United States and four provinces in Canada made cooperative experiments in 1922, 1923, and 1924. Brief progress reports of these investigations were compiled annually (6, 7, and 8). Since the project was begun, many of the cooperators, as well as others, have published the complete results of their investigations. Their results are essentially in agreement with those obtained in the cooperative experiments. However, on account of the scope of the cooperative tests, it seems advisable to publish the results.

COOPERATORS

The following investigators cooperated in the project:

UNITED STATES

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Newark, Del.

Dr. W. H. Martin,
New Jersey Experiment Station,
New Brunswick, N. J.

Dr. C. R. Orton,
Pennsylvania State College,
State College, Penn.

Dr. E. C. Stakman,
Minnesota Agricultural Experiment Station,
St. Paul, Minn., in cooperation with:

Mr. R. S. Dunham,
Northwest School and Station,
Crookston, Minn.

Mr. M. J. Thompson,
Northeast Experiment Station,
Duluth, Minn.

Mr. R. O. Bridgford,
West Central School and Station,
Morris, Minn.

Mr. R. E. Hodgson,
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Waseca, Minn.

Dr. F. D. Heald,
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Prof. George H. Dugan,
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Moscow, Ida.

CANADA

Dr. B. T. Dickson,
MacDonald College,
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Prof. J. E. Howitt,
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Dr. G. R. Bisby,
Manitoba Agricultural College,
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Prof. W. P. Fraser,
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Saskatoon, Sask.

MATERIALS AND METHODS

There was some variation in the use of the different fungicides. It was originally suggested that the following fungicides be used: copper sulphate dip, formaldehyde spray, formaldehyde sprinkle, formaldehyde dip, copper carbonate dust, and dehydrated copper sulphate dust. However, many promising fungicides have been placed on the market since the project was begun in 1922. Several of these were included in the tests, and, in some instances, substituted for those listed above. Only three tests were made with the copper sulphate dip method; the results were inconclusive and have therefore been omitted from this paper. The following fungicides were used: formaldehyde dip, formaldehyde spray, copper sulphate plus lime dust, copper carbonate dust, nickel carbonate dust, Seed-O-San dust, Semesan dust, and solutions of Semesan and of Uspulun.

Many of the cooperators deviated slightly from the original plan of applying the fungicides. These variations apparently had little influence on the results obtained and will therefore not be discussed in detail. The dusts were usually applied at the rate of two ounces a bushel for wheat, and either two or three ounces a bushel for oats and barley. Formaldehyde was used in two strengths: as a dip or sprinkle diluted at the rate of one part of 40 per cent liquor of formaldehyde to 320 parts of water, followed by a one- to five-hour cover; or as a spray, diluted 1 : 1 with water, and applied at the rate of one quart of diluted solution to 50 bushels of grain, as recommended by Haskell (4). Semesan and Uspulun were applied in solution, in strengths of 0.30 per cent and 0.25 per cent, respectively, the period of soak varying from 15 minutes to two hours. Tests also were made with these fungicides after presoaking the grain.

In the original outline of the experiment it was suggested that triplicated one-fortieth acre plots be used. A provision was added that the best agronomic practice of the station at which the tests were made be followed, if it was necessary to deviate from this plan. It was evident from the reports of the cooperators that there had been but little uniformity either in size of plots or number of replications. The size of the plots varied from a single rod row to one-fortieth acre, and the number of replications from one to six. Because of these differences, it was impossible to analyze the data on a statistical basis.

TABLE 1.—*Results of treating wheat with various fungicides at 20 different stations in the United States and Canada in 1922, 1923, and 1924*

Station	Year	Treatments														
		Check			Formaldehyde dip (1–320)			Copper carbonate dust			Copper sulphate plus lime dust			Seed-O-San dust		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Alberta																
Lacombe	1922	100.0	16.9	25.3	90.0	0.0[d]	26.3	82.0	0.6	25.3	90.0	2.2	25.3	.....	.....	.....
	1923	68.0[c]	46.0	33.0	23.0[c]	0.0[d]	48.7	76.0[c]	1.0	51.0	.....	.....	.....	.....	.....	.....
Ontario																
Guelph	1923	91.0[c]	0.0	8.7	4.0[c]	0.0[d]	3.3	88.0[c]	0.0	6.9	86.0[c]	0.0	8.4	.....	.....	.....
Manitoba																
Winnipeg	1923	46.5	2.1	.....	37.0	0.0[d]	.....	41.5	Trace	.....	38.0	1.3	.....	36.0	1.0	.....
Brandon	1924	.....	22.7	.....	.....	1.0	.....	.....	7.3	.....	.....	.....	.....	.....	.....	.....
North Dakota																
Fargo	1923	.....	17.2	12.0[b, e]	.....	0.0[d]	11.2[b, e]	.....	0.0	11.8[b, e]	.....	0.0	11.8[b, e]	.....	0.0	12.3[b, e]
South Dakota																
Brookings	1922	.....	90.0	.....	.....	0.0[d]	.....	.....	0.0	.....	.....	.....	.....	.....	.....	.....
Idaho																
Moscow	1923	94.5	25.0	.....	.....	.....	.....	95.0	0.3	.....	86.0	2.6	.....	.....	.....	.....
	1924	99.0	16.2	52.7[b]	90.0	0.0	45.4[b]	95.5	0.2	58.1[b]	.....	.....	.....	.....	.....	.....
Washington																
Pullman	1923	.....	46.7	.....	.....	0.0[d]	.....	.....	5.9	.....	.....	.....	.....	.....	.....	.....
	1924	.....	36.2	.....	.....	0.0	.....	.....	0.0	.....	.....	.....	.....	.....	.....	.....
Ohio																
Wooster	1923	.....	46.0	.....	.....	.....	.....	.....	0.0	.....	.....	Trace	.....	.....	.....	.....
Illinois																
Urbana	1923	.....	0.0	4.9[b]	.....	0.0[d]	9.0[b]	.....	0.0	7.7[b]	.....	0.0	7.5[b]	.....	0.0	9.9[b]
Minnesota																
St. Paul	1922	.....	0.6	26.3[b]	.....	0.0[d]	20.9[b]	.....	0.0	28.5[b]	.....	.....	.....	.....	0.0	27.8[b]
	1923	87.0	24.3	20.5[b]	87.0	0.0[d]	21.8[b]	94.0	0.7	21.2[b]	.....	.....	.....	93.0	0.0	21.5[b]
	1924	80.0	15.5	28.3[b]	64.0	Trace	24.8[b]	82.0	Trace	34.0[b]	.....	.....	.....	89.0	2.0	34.3[b]
Crookston	1922	.....	11.3	20.6	.....	0.0[d]	17.4	.....	1.6	22.2	.....	.....	.....	.....	0.6	23.7
	1923	65.0	4.2	13.7	87.0	0.0[d]	9.6	94.0	0.0	13.2	.....	.....	.....	93.0	0.0	14.7
	1924	80.0	5.7	32.7[b]	64.0	0.0	25.6[b]	84.5	Trace	37.8[b]	.....	.....	.....	82.0	Trace	35.1[b]

Station	Year	Treatments														
		Check			Formaldehyde dip (1–320)			Copper carbonate dust			Copper sulphate plus lime dust			Seed-O-San dust		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Crookston	1922	.....	11.3	20.6	.....	0.0[d]	17.4	.....	1.6	22.2	.....	.....	.....	.....	0.6	23.7
	1923	65.0	4.2	13.7	87.0	0.0[d]	9.6	94.0	0.0	13.2	.....	.....	.....	93.0	0.0	14.7
	1924	80.0	6.7	32.7[b]	64.0	0.0	25.6[b]	84.5	Trace	37.8[b]	.....	.....	.....	89.0	Trace	35.1[b]
	1924	80.0	15.3	28.3[b]	64.0	Trace	24.8[b]	82.0	Trace	34.0[b]	.....	.....	.....	89.0	2.0	34.3[b]

TABLE 1.—(*Continued*)

Station	Year	Treatments														
		Check			Formaldehyde dip (1–320)			Copper carbonate dust			Copper sulphate plus lime dust			Seed-O-San dust		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota																
Grand Rapids	1922	.....	8.3	21.5	.....	0.0[d]	23.0	.....	0.3	22.3	.....	.....	.....	.....	1.0	22.3
Coon Creek	1924	80.0	13.0	.....	64.0	0.0	.....	84.5	0.0	.....	.....	.....	.....	89.0	Trace	.....
Morris	1922	.....	2.0	15.3	.....	0.0[d]	14.9	.....	0.0	18.2	.....	.....	.....	.....	2.6	17.4
	1923	65.0	2.0	10.8	87.0	0.0[d]	10.5	94.0	0.0	12.0	.....	.....	.....	93.0	0.0	11.6
	1924	80.0	10.7	22.6[b]	64.0	0.0	16.9[b]	84.5	Trace	22.3[b]	.....	.....	.....	89.0	Trace	21.2[b]
Waseca	1922	.....	6.5	22.5	.....	0.0[d]	18.8	.....	0.0	19.5	.....	.....	.....	.....	.....	.....
	1923	65.0	.....	16.3	87.0	.....	14.4	94.0	.....	16.1	.....	.....	.....	93.0	.....	14.1
Saskatchewan																
Indian Head	1922	90.0	5.5	53.7[e]	96.0	0.0[d]	53.3[e]	92.0	0.4	54.0[e]	98.0	0.7	58.7[e]	.....	.....	.....
(Exp. Farm)	1923	68.2	4.0	12.4	23.0	0.0[d]	13.5	76.0	0.0	17.2	75.0	0.0	16.2	.....	.....	.....
Indian Head (Dom. Lab.)	1922	90.0	15.8	.....	96.0	0.0[d]	.....	86.0	1.0	.....	94.0	3.1	.....	.....	.....	.....
Rosthern	1922	98.0	17.7	34.7[e]	94.0	0.0[d]	39.3[e]	100.0	1.3	39.3[e]	96.0	5.0	38.7[e]	.....	.....	.....
	1923	68.0	5.6	21.3	23.0	Trace[d]	21.3	76.0	Trace	22.3	.....	.....	.....	.....	.....	.....
Scott	1922	100.0	5.4	18.2	90.0	0.0[d]	16.3	82.0	0.0	17.3	90.0	Trace	16.6	.....	.....	.....
	1923	68.2	45.0	25.7	23.0	Trace[d]	45.5	76.0	0.7	44.1	75.0	0.5	46.7	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.

[b] Yield tests from rod rows.

[c] Germination in soil.

[d] Formaldehyde sprinkle.

[e] No replication.

The results reported by the various cooperators from 1921 to 1924, inclusive, are summarized in tables 1 to 8. Unless otherwise noted, the figures for yield are the averages for replicated plots, and the figures for percentage of germination are the results of tests made in sand.

RESULTS OF INVESTIGATIONS

Wheat. The data for wheat are given in tables 1 and 2.

The superiority of copper carbonate dust over the other fungicides tried is clearly indicated. It is apparent that, in nearly all of the tests, the various fungicides, with the exception of formaldehyde, had little or no effect on the germination of the seed. Numerous investigators reported apparent seed injury from the use of formaldehyde. At Guelph, Ontario, in 1923, germination was reduced from 99 per cent in the check to 4 per cent in the treated plot. Likewise, in the same year at Indian Head, Saskatchewan, formaldehyde apparently reduced germination from 68 per cent in the check to 23 per cent in the formaldehyde treated plot. Formaldehdye did not injure the seed at Indian Head, Saskatchewan, in 1922, nor at University Farm, St. Paul, Minnesota, in 1923. Some of the minor differences in germination may have been due to random sampling in poor seedlots.

Several of the fungicides effectively controlled bunt. Of these, formaldehyde was the most effective. At only one station did as much as 1 per cent of bunt develop in the formaldehyde plots. Copper carbonate dust controlled bunt satisfactorily in nearly all of the trials. It reduced the amount of bunt to less than 2 per cent, except in two trials. At Pullman, Washington, 5.9 per cent developed in 1923; and at Brandon, Manitoba, 7.33 per cent was recorded in 1924. In most of the tests, however, the bunt was almost entirely eliminated. The copper sulphate plus lime dust consistently reduced the amount of bunt but not sufficiently to justify its recommendation for practical use. Seed-O-San, nickel carbonate, and Semesan, applied as dusts, reduced the amount of smut, Semesan apparently being the most effective. Solutions of the organic mercury compounds, Semesan and Uspulun, were tested, but the results were somewhat conflicting. In 1924, Uspulun solution completely eliminated bunt of wheat at University Farm, Minnesota, and at Moscow, Idaho; but 1.83 per cent of bunt developed at Brandon, Manitoba. Semesan, when applied in solution, was effective in Minnesota and North Dakota; but 5.33 per cent and 2.07 per cent of bunt developed in treated plots at Brandon, Manitoba, and Moscow, Idaho, respectively.

Yield tests with wheat were inconclusive. There were twenty-four experiments in which yield comparisons were made between check plots and plots for seed treated with formaldehyde, and with copper carbonate dust. The plots from copper carbonate treated seed yielded more than the checks

TABLE 2.—*Results of treating wheat with various fungicides at 10 different stations in the United States and Canada in 1923 and 1924*

Station	Year	Treatments														
		Check			Nickel carbonate			Semesan soak 0.3 per cent			Semesan dust			Uspulun 0.25 per cent		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota																
St. Paul	1923	87.0	24.3	20.5[b]	92.0	2.3	23.3[b]	.....	.....	.....	.....	0.0	23.5[b]	.....	.....	.....
	1924	80.0	15.5	28.3[b]	76.5	4.0	33.5[b]	89.0	Trace	31.9[b]	86.5	Trace	26.7[b]	89.5	0.0	33.3[b]
Coon Creek	1924	80.0	13.0								86.5	Trace	.....	.....	.....	.....
Crookston	1924	80.0	6.7	22.7[b]	.....	.....	.....	.....	.....	.....	86.5	1.2	22.0	.....	.....	.....
Morris	1924	80.0	10.7	22.6[b]	.....	.....	.....	.....	.....	.....	86.5	Trace	21.9[b]	.....	.....	.....
Manitoba																
Winnipeg	1923	46.5	2.1	.....	44.5	3.5	.....	73.4	0.5	.....	.....	.....	.....	.....	.....	.....
Brandon	1924	.....	22.7	.....	.....	12.3	.....	.....	5.3	.....	.....	2.0	.....	.....	1.8	.....
Idaho																
Moscow	1923	94.5	25.0	.....	93.0	2.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	1924	99.0	16.2	52.7[b]	93.0	0.0	51.2[b]	96.0	2.1	52.7[b]	98.0	Trace	48.3[b]	100.0	0.0	55.6[b]
Washington																
Pullman	1923	.....	46.7	.....	.....	11.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	1924	.....	36.2	.....	.....	.....	.....	.....	.....	.....	.....	0.6	.....	.....	.....	.....
Ohio																
Wooster	1923	.....	46.0	.....	.....	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
North Dakota																
Fargo	1923	.....	17.2	12.1[b]	.....	0.0	13.0[b]	.....	0.0	12.0[b]	.....	0.0	13.5[b]	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.

[b] Yield tests from rod rows.

in seventeen of the twenty-four experiments and less than the checks in seven experiments. The formaldehyde plots yielded more than the check plots in nine experiments, less than the checks in fourteen experiments, and in one case the yield was identical with that of the check.

Hulled Oats. The data for hulled oats are given in tables 3 and 4.

None of the treatments seemed to have any consistent effect on seed germination of hulled oats: there was considerable fluctuation in the percentages of germination. In some cases treated seed did not germinate as well as untreated seed, while in others the reverse was true. It was impossible to determine from single tests whether these differences were due to random sampling or to the effect of the treatments. Experiments in which numerous check plots were planted proved this conclusively; for example, at Winnipeg in 1923 the average germination of untreated seed was 95 per cent, while the percentages for seed treated with formaldehyde and with copper carbonate were 84 and 66 respectively (table 3). These results might lead one to the conclusion that formaldehyde reduced germination 11 per cent, and that copper carbonate reduced it 29 per cent, but there were greater differences than this in the germination of replicated checks, and the apparent injury of treated seed was probably due to random sampling, as the percentage of germination of 16 random samples of untreated seed varied from 59 per cent to 100 per cent.

Formaldehyde was by far the most effective of the fungicides used for controlling smuts of oats, and was the only one which could be considered as entirely satisfactory. The other substances did not consistently reduce the percentage of smut. Seed-O-San markedly reduced the percentage of smut only at Fargo, North Dakota, in 1923. In three tests, at Winnipeg, Manitoba, in 1923, and at Wooster, Ohio, in both 1923 and 1924, seed treated with Seed-O-San produced more smut than did the untreated seed. Copper carbonate was the most effective of the dusts, although it did not control smut satisfactorily. In all cases it reduced the percentage of smut, but there was considerable difference in the extent to which it controlled the smuts in the experiments of different cooperators (table 3). Some of these differences in smut control may have been due to the fact that copper carbonate often is less effective on naturally inoculated seed than on that which has been artificially inoculated. Dr. R. S. Kirby emphasized this point in the conclusion of his 1923 report. He states: "Apparently the effectiveness of the various treatments can be determined only by the use of naturally inoculated seed. Artificial inoculation, when accomplished by mixing the seed with dry smut spores, does not allow the spores to get inside the glumes, or for them to germinate and form sporidia inside the glumes, as pointed out by Zade. This was shown in the 1923 experiments at Ithaca,

where the sulphur and copper carbonate dusts and Chlorophol steep treatments gave perfect control when artificially inoculated seed was used but gave only fair to moderate control when tested out on naturally inoculated seed."

Although nickel carbonate and copper sulphate plus lime dust reduced the smut in all trials, they usually were less effective than copper carbonate. The organic mercury compounds were unsatisfactory in practically all cases. Copper carbonate and nickel carbonate were the most promising dusts, while formaldehyde was distinctly better than solutions of Semesan and Uspulun.

Apparently none of the treatments consistently increased or decreased the yield. In most cases treated seed yielded more than the untreated, although the reverse was true in a few cases. This variation may have been due to soil heterogeneity. The only plots in which yields were determined, where the check plots were heavily smutted, were at St. Paul, Minnesota, in 1923. There the treated plots yielded considerably more than the checks and the difference in yield was roughly proportional to the degree of smut control.

Hulless Oats. The data for hulless oats are given in tables 5 and 6.

The tests with hulless oats were made at only a few stations—all in Canada—and fewer fungicides were used than for wheat and hulled oats. The results of germination tests, smut counts, and yield tests indicate that copper carbonate was the best of the fungicides tested. Formaldehyde was decidedly injurious to seed, while copper carbonate and copper sulphate plus lime dusts did not seem to affect seed germination. The dusts controlled the smut effectively in all of the tests. In two of them, formaldehyde reduced the percentage of smut only slightly and, in general, was not so effective as copper carbonate.

Nearly all of the treated plots yielded more than the checks. This was especially noticeable when the yields of copper carbonate plots were compared with those of badly smutted check plots. In some cases the treated plots yielded two or three times as much as the checks. For example, at Scott, Saskatchewan, in 1923, the check plot contained 62 per cent of smut and yielded 21.8 bushels an acre, while the copper carbonate plot was free from smut and yielded 52.8 bushels an acre. And at MacDonald College, Quebec, in 1922, the check plot contained 49 per cent of smut and yielded 12.5 bushels an acre, while the copper carbonate plots contained 0.3 per cent of smut and yielded 28 bushels an acre. Again, at MacDonald College, in 1923, the check contained 71.4 per cent of smut and yielded 12.3 bushels an acre, while the copper carbonate plot contained 1.5 per cent of smut and yielded 36.6 bushels an acre.

TABLE 3.—*Results of treating oats with various fungicides at 20 different stations in the United States and Canada in 1922, 1923, and 1924*

Station	Year	Treatments														
		Check			Formaldehyde dip (1–320)			Copper carbonate dust			Copper sulphate plus lime dust			Seed-O-San dust		
		Pet. germ.	Pet. smut	Acre[a] yields	Pet. germ.	Pet. smut	Acre[a] yields	Pet. germ.	Pet. smut	Acre[a] yields	Pet. germ.	Pet. smut	Acre[a] yields	Pet. germ.	Pet. smut	Acre[a] yields
Minnesota																
St. Paul	1922	.....	1.0	55.5[b]	.....	0.0	48.4[b]	.....	0.0	51.1[b]	.....	.....	.....	.....	Trace	63.8[b]
	1923	83.0	38.7	47.6[b]	96.0	Trace	68.8[b]	92.0	6.0	60.0[b]	.....	.....	.....	98.0	19.6	57.0[b]
	1924	91.5	0.0	56.3[b]	79.0	0.0	62.1	91.5	0.0	58.9[b]	.....	.....	.....	84.0	0.0	39.0[b]
Crookston	1922	.....	Trace	77.0	.....	0.0	69.1	.....	Trace	81.3	.....	.....	.....	.....	Trace	72.6
	1923	83.0	10.1	49.9	96.0	0.0	48.4	92.0	7.6	53.9	.....	.....	.....	98.0	10.8	51.8
	1924	91.5	1.5	74.8[b]	79.0	0.0	76.8[b]	91.5	Trace	71.4[b]	.....	.....	.....	84.0	Trace	61.4[b]
Duluth	1922	.....	1.6	48.9	.....	Trace	38.5	.....	Trace	55.5	.....	.....	.....	.....	.....	.....
	1923	.....	3.7	56.8	.....	0.6	51.0	.....	0.8	55.6	.....	.....	.....	.....	2.3	55.0
Coon Creek	1924	91.5	7.0	.....	79.0	0.0	.....	91.5	5.0	.....	.....	.....	.....	84.0	6.3	.....
Waseca	1922	.....	2.5	55.1	.....	0.0	55.1	.....	Trace	52.0	.....	.....	.....	.....	.....	.....
	1923	.....	.....	46.2	.....	.....	42.3	.....	.....	48.9	.....	.....	.....	.....	.....	32.7
Morris	1924	91.5	5.3	71.8[b]	79.0	0.0	76.7[b]	91.5	2.3	68.4[b]	.....	.....	.....	84.0	3.3	72.9[b]
Saskatchewan																
Indian Head	1922	100.0	7.5	.....	94.0	0.0	.....	100.0	1.0	.....	100.0	5.6	.....	.....	.....	.....
	1923	.....	22.4	.....	.....	0.0	.....	.....	0.4	.....	.....	0.8	.....	.....	.....	.....
Ontario																
Guelph	1922	.....	2.2	76.0	.....	Trace[d] / 0.0[e]	76.1[d] / 74.6[e]	.....	1.4	77.3	.....	2.0	78.6	.....	.....	.....
	1923	98.0[e]	4.4	44.5	46.0[d] / 98.0[e]	Trace[d] / Trace[e]	36.9[d] / 46.0[e]	97.0[e]	1.2	44.8	94.0[e]	3.1	44.6	.....	.....	.....
	1924	95.0	1.3	.....	82.0[d] / 95.0[e]	0.0[d] / 0.0[e]	.....	.....	.....	.....	.....	.....	.....	93.0	0.3	.....
Quebec																
MacDonald College	1922	94.0	1.5	61.8[g]	.....	.....	.....	91.0	0.0	63.2[g]	.....	.....	.....	.....	.....	.....
Manitoba																
Winnipeg	1923	95.0	5.8	18.8[b]	84.0[d] / 84.0[e]	0.3[d] / 0.0[e]	21.4[d, b] / 18.1[e, b]	66.0	0.0	19.4[b]	81.5	Trace	20.0[b]	95.5	8.2	18.2[b]

Manitoba																
Winnipeg	1923	95.0	5.8	18.8[b]	84.0[d] / 84.0[e]	0.3[d] / 0.0[e]	21.4[d, b] / 18.1[e, b]	66.0	0.0	19.4[b]	81.5	Trace	20.0[b]	95.5	8.2	18.2[b]

TABLE 3.—(*Continued*)

Station	Year	Treatments														
		Check			Formaldehyde dip (1–320)			Copper carbonate dust			Copper sulphate plus lime dust			Seed-O-San dust		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ	Pct. smut	Acre[a] yields
North Dakota																
Fargo	1923	.....	5.1	45.2[b]	.....	0.0[d] / 0.0[e]	46.9[d, b] / 4.97[e, b]	.....	3.0	42.2[b]	.....	0.0	31.4[b]	.....	0.0	47.3[b]
Illinois																
Urbana	1922	.....	2.2	.....	.....	0.0	.....	.....	Trace	.....	.....	.....	.....	.....	.....	.....
	1923	.....	4.0	9.9[b]	.....	0.0[d] / 0.0[e]	12.5[d, b] / 11.1[e, b]	.....	1.8	11.5[b]	.....	1.9	11.5[b]	.....	3.8	12.7[b]
Ohio																
Wooster	1923	.....	2.1	.....	.....	0.8[d]	.....	.....	1.3	.....	.....	.....	.....	.....	3.6	.....
	1924	97.0[f]	35.6	.....	97.0[f]	0.0[d]	.....	96.0[f]	4.3	.....	.....	.....	.....	87.0	36.7[f]	.....
Pennsylvania																
State College	1922	.....	7.2	.....	.....	.....	.....	.....	3.6	.....	.....	.....	.....	.....	.....	.....
	1923	92.0	8.0	22.0[b]	85.0	0.0	.....	96.0	3.0	23.6[b]	.....	.....	.....	.....	.....	.....
	1924	88.0[f]	7.2	22.7[b]	88.0[f]	0.0	26.0[b]	78.0[f]	1.2	25.7[b]	.....	.....	.....	.....	.....	.....
New Jersey																
New Brunswick	1923	93.3	4.1	24.6[b]	96.3[d] / 96.3[e]	0.2[d] / 0.3[e]	25.1[d, b] / 25.9[e, b]	97.0	0.9	25.4[b]	96.6	1.2	22.9[b]	.....	.....	.....
	1924	100.0	4.1	35.1[b]	55.0	0.0	30.6[b]	98.0	0.4	36.0[b]	.....	.....	.....	.....	.....	.....
Delaware																
Newark	1922	.....	4.1	.....	.....	0.0[e]	.....	.....	Trace	.....	.....	2.5	.....	.....	.....	.....
	1923	.....	4.7	.....	.....	Trace	.....	.....	0.5	.....	.....	.....	.....	.....	.....	.....
Washington																
Pullman	1923	.....	30.0	73.0	.....	1.5	71.6	.....	1.0	79.1	.....	.....	.....	.....	.....	.....
Idaho																
Moscow	1924	100.0	16.0	.....	99.0	Trace	.....	100.0	1.8	.....	.....	.....	.....	.....	.....	.....
New York																
Ithaca	1923	.....	8.5	.....	.....	0.4[e]	.....	.....	1.5	.....	.....	.....	.....	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.
[b] Yield tests from rod rows.
[c] Germination in soil.
[d] Formaldehyde sprinkle.
[e] Formaldehyde spray.
[f] Germination on blotter.
[g] Yield tests on one-hundredth acre plots. No replications.

TABLE 4.—*Results of treating hulled oats with various fungicides at 14 different stations in the United States and Canada in 1922, 1923 and 1924*

Station	Year	Treatments														
		Check			Nickel carbonate			Semesan dust			Semesan soak 0.3 per cent			Uspulun 0.25 per cent		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota																
St. Paul	1923	83.0	38.7	47.6[b]	94.0	9.7	49.3[b]	89.0	17.7	63.2[b]	.....	.....	.....	.....	.....	.....
	1924	91.5	0.0	56.3[b]	90.5	0.0	53.1[b]	91.5	0.0	54.2[b]	96.5	0.0	55.2[b]	96.5	0.0	55.9[b]
Coon Creek	1924	91.5	7.0	.....	.....	.....	.....	91.5	5.7	.....	.....	.....	.....	.....	.....	.....
Crookston	1924	91.5	1.5	74.8[b]	.....	.....	.....	91.5	Trace	58.8[b]	.....	.....	.....	.....	.....	.....
Morris	1924	91.5	5.3	71.8[b]	.....	.....	.....	91.5	1.7	71.5[b]	.....	.....	.....	.....	.....	.....
Manitoba																
Winnipeg	1923	95.0	5.8	18.8[b]	82.5	2.7	19.0[b]	.....	.....	.....	87.5	1.1	19.6[b]	.....	.....	.....
Brandon	1924	.....	13.1	.....	.....	0.2	.....	.....	4.2	.....	.....	5.7	.....	.....	2.0	.....
Ontario																
Guelph	1923	98.0[c]	4.4	44.5	95.0[c]	2.4	40.2	.....	.....	.....	.....	.....	.....	.....	.....	.....
	1924	95.0	1.3	.....	93.0	Trace	.....	94.0	0.3	.....	91.0	Trace	.....	90.0	Trace	.....
North Dakota																
Fargo	1923	.....	5.1	45.2[b]	.....	0.0	35.7[b]	.....	0.0	45.9[b]	.....	0.0	45.0[b]	.....	.....	.....
Illinois																
Urbana	1923	.....	4.0	9.9[b]	.....	1.7	8.5[b]	.....	.....	.....	.....	.....	.....	.....	.....	.....
New Jersey																
New Brunswick	1923	93.3	4.1	24.6[b]	98.0	0.2	26.4[b]	98.3	3.8	25.7[b]	.....	.....	.....	.....	.....	.....
	1924	100.0	4.1	35.1[b]	100.0	0.2	35.9[b]	98.0	1.3	34.6[b]	100.0	0.4	36.8[b]	100.0	0.2	35.8[b]
Pennsylvania																
State College	1923	92.0	8.0	22.0[b]	94.0	0.5	25.6[b]	93.0	8.0	23.6[b]	.....	.....	.....	.....	.....	.....
	1924	88.0[d]	7.2	22.7[b]	92.0[d]	1.1	25.9[b]	90.0[d]	6.3	25.4[b]	88.0[d]	4.1	25.8[b]	.....	2.6	26.3[b]
Ohio																
Wooster	1923	.....	2.1	.....	.....	0.0	.....	.....	2.0	.....	.....	2.1	.....	.....	.....	.....
	1924	97.0[d]	35.6	.....	96.0[d]	3.6	.....	92.0[d]	23.5	.....	92.0[d]	12.2	.....	96.0[d]	6.3	.....
Idaho																
Moscow	1924	100.0	16.0	.....	99.0	9.4	.....	100.0	9.7	.....	100.0	9.1	.....	100.0	6.6	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.
[b] Yield tests from rod rows.
[c] Germination in soil.
[d] Germination in blotter.

TABLE 5.—*The results of treating hulled*

[a] Yield tests from one-fortieth acre plots except as otherwise noted.
[b] Yield tests from rod rows.
[c] Germination in soil.
[d] Germination in blotter.

TABLE 5.—*The results of treating hulless oats with various fungicides at seven stations in Canada in 1922, 1923 and 1924*

Station	Year	Treatments											
		Check			Formaldehyde dip (1–320)			Copper carbonate			Copper sulphate plus lime dust		
		Pct.[b] germ.	Pct. smut	Acres[a] yields	Pct.[b] germ.	Pct. smut	Acres[a] yields	Pct.[b] germ.	Pct. smut	Acres[a] yields	Pct.[b] germ.	Pct. smut	Acres[a] yields
Saskatchewan													
Indian Head	1923	71.5	20.1	25.4	33.5	13.8[c]	25.8	69.0	0.8	28.1	84.0	0.4	31.4
Scott	1923	57.0	62.5	21.8	17.5	1.6[c]	50.2	51.5	0.0	52.8	.....	.....	.....
Rosthern	1923	70.5	22.0	40.3	33.0	5.9[c]	38.9	70.5	Trace	44.6	.....	.....	.....
Alberta													
Lacombe	1923	59.5	35.3	41.3	16.5	1.9[c]	52.8	46.0	Trace	55.6	.....	.....	.....
Manitoba													
Brandon	1924	.....	52.1	.....	.....	14.9[c] / 42.3	.....	.....	3.3	.....	.....	.....	.....
Quebec													
MacDonald College	1922	82.0	49.0	12.5[e]	65.0[c] / 23.0[d]	1.0[c] / 0.3[d]	14.0[c, e] / 16.0[d, e]	90.0	0.3	28.0[e]	89.0	0.3	28.0[e]
	1923	93.0	71.4	12.3	77.0[c] / 0.7[d]	1.8[c]	33.5[e]	93.0	1.5	36.6	80.0	1.5	38.6
	1924	.....	58.4	.....	.....	3.3	.....	.....	2.1	.....	.....	.....	.....
Ontario													
Guelph	1922	.....	4.5	15.3[f]	.....	.....	.....	.....	0.2	15.7[f]	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.
[b] Germination in soil.
[c] Formaldehyde sprinkle.
[d] Formaldehyde spray.
[e] Yield tests on one-hundredth acre plots. No replications.
[f] No replications.

TABLE 6.—*Results of treating hulless oats with various fungicides at two stations in Canada in 1924*

Station	Year	Treatments													
		Check			Nickel carbonate			Semesan dust			Semesan soak 0.3 per cent			Uspulun 0.25 per cent		
		Pct. germ.	Pct. smut	Acre yields	Pct. germ.	Pct. smut	Acre yields	Pct. germ.	Pct. smut	Acre yields	Pct. germ.	Pct. smut	Acre yields	Pct. germ.	Pct. smut	Acre yields
Quebec																
MacDonald College	1924	.....	58.4	.....	.....	3.8	.....	.....	6.0	.....	.....	6.4	.....	.....	3.4	.....
Manitoba																
Brandon	1924	.....	52.1	.....	.....	3.4	.....	.....	14.3	.....	.....	16.4	.....	.....	11.3	.....

Barley. The data for barley are given in tables 7 and 8.

The tests with barley were not as extensive as those with either wheat or oats. In most cases they were not conclusive because of the small amount of smut in the checks. There seemed to be little or no seed injury as a result of any of the treatments. Many of the treatments reduced the amount of smut, but formaldehyde was the only fungicide that consistently controlled it. The yield tests also were inconclusive.

DISCUSSION AND CONCLUSIONS

The advantages of dusts are obvious. However, none of those tested controlled the smuts of hulled oats and barley as satisfactorily as did the liquids. It therefore seems necessary to recommend a liquid treatment for hulled grain.

Copper carbonate dust seems to be the best fungicide for wheat and hulless oats, whereas formaldehyde is most effective for hulled oats and barley. Copper carbonate controls bunt of wheat and the smuts of hulless oats cheaply and effectively without injuring the seed. In addition, it has the advantage of being applied as a dust. The formaldehyde and copper sulphate treatments control the smuts of these grains, but they wet the seed and often injure the seed.

Formaldehyde controlled the smuts of hulled oats more satisfactorily than any of the other materials tested. The formaldehyde spray method, recommended by Haskell (4), seems to be the most desirable for oats. This treatment is too injurious for use on wheat or hulless oats, but apparently it is safe for hulled oats and has the advantage of being practically a dry treatment, as the seed does not become wet if the formaldehyde is applied properly.

None of the dusts consistently controlled smuts of oats. Copper carbonate, which was the most effective of them, eliminated the smuts completely in some tests but not in others. Recent investigations by Zade (9) suggest plausible explanations for the disagreement in the results obtained by different cooperators. He found that *Ustilago avenae* persists from one crop year to the next in the form of mycelium and chlamydospores under the glumes and in the epidermis of the caryopsis. Thus the disparity in the results obtained by different investigators may have been due to the fact that some used naturally inoculated seed, while others used artificially inoculated seed. On the other hand, it is possible that copper carbonate dust controls the covered smut but not the loose smut, and that the difference in the effectiveness of the dusts in the different experiments was due to the amount of loose smut present in the seedlots.

TABLE 7.—*Results of treating barley with various fungicides at 10 stations in the United States and Canada in 1922, 1923, and 1924*

Station	Year	Treatments													
		Check			Formaldehyde dip (1–320)			Copper carbonate			Copper sulphate plus lime dust			Seed-O-San		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota																
St. Paul	1922	.....	0.0	61.2[b]	.....	0.0[c]	65.3[b]	.....	0.0	60.8[b]	.....	.....	.....	.....	0.0	60.0[b]
	1923	81.0	1.3	47.7[b]	76.0	0.0[c]	43.7[b]	83.0	1.0	49.6[b]	.....	.....	.....	87.0	0.8	42.4[b]
	1924	90.3	0.0	32.7[b]	88.0	0.0	31.8[b]	92.5	0.0	31.2[b]	.....	.....	.....	89.5	0.0	31.0[b]
Crookston	1922	.....	1.3	48.6	.....	0.0[c]	62.6	.....	0.6	62.3	.....	.....	.....	.....	0.6	46.8
	1923	72.5	3.5	31.4	76.0	Trace	40.3	83.0	0.7	40.7	.....	.....	.....	87.0	1.5	39.8
	1924	90.3	0.0	48.6[b]	88.0	0.0	53.1[b]	92.5	0.0	41.7[b]	.....	.....	.....	89.5	0.0	44.3[b]
Coon Creek	1924	90.3	3.3	20.5[b]	88.0	1.3	20.5[b]	92.5	0.8	22.2[b]	.....	.....	.....	89.5	2.7	23.8[b]
Morris	1922	.....	0.0	38.1	.....	0.0[c]	44.4	.....	0.0	41.2	.....	.....	.....	.....	0.0	43.0
	1923	72.5	5.3	27.9	76.0	Trace	27.4	83.0	4.0	29.3	.....	.....	.....	87.0	4.3	29.7
	1924	90.3	2.3	18.5[b]	88.0	Trace	17.8[b]	92.5	1.0	18.3[b]	.....	.....	.....	89.5	2.3	20.3[b]
Waseca	1923	72.5	.....	11.7	76.0	.....	10.7	8.3	.....	15.0	.....	.....	.....	87.0	.....	10.6
Saskatchewan																
Indian Head	1923	.....	6.6[d]	.....	.....	0.0[d, e]	.....	.....	2.0[d]	.....	.....	.....	.....	.....	.....	.....
Manitoba																
Brandon	1924	.....	7.9	.....	.....	3.8 / 2.7[e]	.....	.....	3.6	.....	.....	.....	.....	.....	.....	.....
North Dakota																
Fargo	1923	.....	Trace	35.9[b]	.....	0.0[c]	38.1[b]	.....	0.0	30.3[b]	.....	0.0	28.7[b]	.....	0.0	32.8[b]
Illinois																
Urbana	1923	.....	0.4	13.4[b]	.....	0.0[c]	9.3[b]	.....	0.0	12.3[b]	.....	0.7	15.3[b]	.....	0.0	12.5[b]
New York																
Livingston Co.	1923	.....	4.2	.....	.....	0.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

[a] Yield tests from one-fortieth acre plots except as otherwise noted.
[b] Yield tests from rod rows.
[c] Formaldehyde applied.

[a] Yield tests from one-fortieth acre plots except as otherwise noted.
[b] Yield tests from rod rows.

TABLE 8.—*Results of treating barley with various fungicides at seven stations in the United States and Canada in 1923 and 1924*

Station	Year	Treatments													
		Check			Nickel carbonate			Semesan dust			Semesan soak 0.3 per cent			Uspulun 0.25 per cent		
		Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields	Pct. germ.	Pct. smut	Acre[a] yields
Minnesota															
St. Paul	1923	81.0	1.3	47.7	79.0	2.2	46.4	87.0	1.0	53.3	.....	.....	.....	.....	.....	.....
	1924	90.3	0.0	32.7	96.5	0.0	32.4	90.5	0.0	34.3	93.0	0.0	33.8	91.5	0.0	32.4
Coon Creek	1924	90.3	3.3	20.5	.....	.....	.....	90.5	1.7	23.0	.....	.....	.....	.....	.....	.....
Morris	1924	90.3	2.3	18.5	.....	.....	.....	90.5	0.7	16.5	.....	.....	.....	.....	.....	.....
Crookston	1924	90.3	.....	48.6	.....	.....	.....	90.5	.....	48.3	.....	.....	.....	.....	.....	.....
North Dakota															
Fargo	1923	.....	Trace	35.9	.....	0.0	30.0	.....	Trace	43.8	.....	0.0	39.1	.....	.....	.....
Illinois															
Urbana	1923	.....	0.4	13.4	.....	0.4	17.5	.....	.....	.....	.....	.....	.....	.....	.....	.....
Manitoba															
Brandon	1924	.....	7.9	.....	.....	1.9	.....	.....	6.2	.....	.....	7.8	.....	.....	2.3	.....

[a] Yield tests from rod rows.

None of the tests with barley were conclusive because there was very little smut in the check plots. However, formaldehyde seemed to control the covered smut most effectively.

It is interesting to note that the most satisfactory treatments, the copper carbonate dust method and the formaldehyde spray method, both were devised in the past decade. New materials are being produced and offered to the experiment stations for trial, usually under trade names, and some of them may prove to be better than those now recommended. However, copper carbonate dust for wheat and hulless oats, and the formaldehyde spray for hulled oats are effective and simple treatments. If new fungicides replace these materials, it would seem that they must do so by their ability to increase yields distinctly, or to control seed- and soil-borne organisms in addition to the covered smuts.

The necessity of making comparative field tests of the value of different fungicides under many different soil conditions, and of carefully considering the effect of the fungicides on seed germination and on yields, cannot be over-emphasized. Laboratory indices of the effect of fungicides on pathogenes and on seed germination, as suggested by Gassner (2 and 3), may be used advantageously to show which chemicals deserve further test, but such tests must be supplemented by field experiments. Furthermore, clean seed is often treated, and therefore the relative effect of fungicides on yield of clean seed must be determined as well as their effect on the yields of badly smutted seed. Germination and yield tests must be made in such a manner as to make possible a statistical analysis of the results; otherwise they will be practically worthless.

SUMMARY

1. This paper summarizes the results of a cooperative project carried out in eleven states in the United States and four provinces in Canada during 1922, 1923, and 1924.

2. The primary object of the project was to compare the general effectiveness of the copper carbonate dust treatment with the standard formaldehyde treatment on the amount of smut and on seed germination and yield of wheat, oats, and barley. Several other materials were tested: nickel carbonate dust, copper sulphate plus lime dust, Semesan dust, and solutions of Uspulun and Semesan.

3. Copper carbonate dust was the most satisfactory of the materials tested for controlling bunt of wheat and smuts of hulless oats. It did not injure the seed appreciably, practically eliminated the smuts, and seemed to have a tendency to increase yields. Formaldehyde controlled the smuts of these cereals more completely than any of the other materials tested, but

often seemed to injure the seed. It was especially injurious to hulless oats. Solutions of Uspulun and Semesan were not as effective as formaldehyde.

4. Haskell's formaldehyde spray method seemed to be the best treatment for hulled oats. Formaldehyde controlled the smuts of hulled oats more completely than any of the other fungicides. Copper carbonate was the best of the dusts but was much less effective on hulled oats than formaldehyde.

5. The experiments with barley were somewhat inconclusive owing to the small percentages of smut in the check plots, but formaldehyde seemed to control the covered smut better than any of the other treatments. Apparently none of the treatments injured barley seed.

6. In most cases no definite conclusions could be drawn regarding the effect of the different treatments on yields, owing to the lack of systematic replication of the experimental plots. However, the results obtained from treating badly smutted hulless oats with copper carbonate dust were so consistent and outstanding that the treatment undoubtedly was at least partially responsible for the increased yield.

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STUDIES ON LEAFHOPPER INJURY TO APPLE LEAVES[1]

A. A. Granovsky[2]

With Plate XXIV

During the past three years, in many orchards at Sturgeon Bay and other points of the Peninsular region of Wisconsin, a peculiar yellowing of the marginal areas of apple leaves was a common occurrence. The symptoms in various degrees of intensity were observed on younger, as well as older, leaves, and could not be ascribed to any well known cause. This yellowing was often noted in association with curling of the leaves caused by aphids and leafhoppers commonly found on apple trees.

Observations in numerous orchards showed that the three species of the leaf-feeding apple aphids were present every year in abundance, infesting the branches, terminal shoots, fruit spurs and water sprouts. The green apple aphid, *Aphis pomi* De Geer, was found to feed largely on rapidly growing tips and water sprouts. The apple grain aphid, *Rhopalosiphum prunifoliae* (Fitch), formerly known as *Aphis avenae*, was confined to apple trees only in the spring and fall, spending its summer on grains and grasses. The rosy apple aphid, *Anuraphis roseus* Baker, commonly known in this country as *Aphis sorbi*, selects its feeding places on the leaves of terminal shoots and fruit spurs, usually on the lower part of the apple tree, until midsummer, when it migrates to the narrow-leaved plantain and returns to the apple trees in the fall.

It was further observed that the leafhoppers of cosmopolitan food habits were also present on apple trees in abundance. They were feeding not only on aphid infested branches, but were often found mixed on the same leaves with the aphids. The following species of leafhoppers were collected in such association: the apple leafhopper, *Empoasca maligna* (Walsh), formerly *E. unicolor* Gillette; the potato leafhopper, *Empoasca fabae* (Harris), commonly known as *E. mali* (Le Baron); a species known as *Empoasca flavescens* (Fabricius), which is closely related to the last named species in structure and in food habits; and the rose leafhopper, *Typhlocyba rosae* (Linnaeus), usually called *Empoa rosae* (Linn.). All the above listed species were observed feeding and breeding on the apple foliage.

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Occasionally the aster leafhopper, *Cicadula sexnotata* (Fallen), was collected. This species was always found in the mature stage, while other species were observed also in nymphal stages.

SYMPTOMS

The typical symptoms in question manifest themselves in a characteristic marginal yellow discoloration of the leaves. It usually begins at the tip of a leaf blade and gradually progresses downward along the midrib and lateral veins in more or less triangular areas at the outer margin of the leaf. This marginal discoloration, when viewed from the upper side of the leaf blade, is invariably more rapid on the proximal side of the lateral veinlets than on the distal side of these veins (Fig. 1). As the symptoms progress, the marginal yellow triangular areas running towards the midrib increase in size and occasionally may reach the midrib. In cases of severe injury, the tips, as well as the distal margins of the leaves, turn brown, showing a characteristic burning. In many instances leaf margins thus affected curled upward, much resembling the hopperburn injury of potatoes (Fig. 2). Although both older and younger leaves are subject to the symptoms, this is especially true of the younger leaves of rapidly growing terminal shoots and the succulent growth of water sprouts. A considerable pocketing and a characteristic downward rolling of the younger leaves was also observed, while the older leaves had only the discoloration symptoms described above without much downward rolling.

With the advent of cold weather the yellow discoloration at first assumed a reddish color which was very prominent, and later it gradually changed to a purple tinge. The normal and unaffected leaves remained green until the first killing frost. Not only were the affected triangular areas between the lateral veinlets changed in color by the approach of cold weather, but the veins and midribs of the affected leaves showed the purplish discoloration far beyond the farthest advance of the triangular discoloration, indicating the possible presence of some infective principle in the vascular system. The progressive downward discoloration along the veins suggests its presence in the phloem region. This, in its turn, suggested the possible insect relation to the symptoms described, as it is illustrated by Woods (19), Davidson (9), and Horsfall (15), that the sucking insects derive their food from the phloem vessels of the vascular bundles.

THE ASSOCIATION OF INSECTS WITH SYMPTOMS

Since these symptoms were found in the field on the leaves infested with the aphids and leafhoppers, these insects were suspected to have some causal

relationship. Yet it will not be amiss to mention that the symptoms are decidedly different from the feeding injury commonly ascribed to these insects. Several entomological workers reported a severe feeding injury to apple trees, especially to nursery stock, both by the leafhoppers and aphids. All of these insects retard the growth of the terminal shoots and nursery stock by extracting the plant juices.

So far as the leaf symptoms are concerned, Lathrop (16) and Ball (7) note that the apple leafhopper, *Empoasca maligna*, and the rose leafhopper, *Typhlocyba rosae*, produce only white stippling and spotting on the upper surface of the older leaves without curling them. The potato leafhopper, *Empoasca fabae*, is feeding on the younger leaves of tips, water sprouts and nursery stock, causing a characteristic downward curling and distortion of the foliage. The tips and margins of badly affected foliage turn brown and dry up without white spotting. Similar description of the feeding injury by *Empoasca fabae* is made by Ackerman (1) as well as others.

No reference to a triangular discoloration along the lateral veinlets and marginal upturn of the leaves is made, and, as far as the writer is aware, there is no definite record of these symptoms in literature on the subject.

In discussing the potato hopperburn, the apple plant is usually listed as a food of leafhoppers, and only a few brief notes [Ball (4, 5, 6), Parrott and Olmstead (18), Fenton and Hartzell (14) and others] mention the fact that leafhoppers may cause curling and tipburn. Only in two instances, Dudley and Wilson (10) and Dudley (11), we find that, in connection with potato hopperburn studies, when the potato leafhoppers were allowed to feed on a few apple seedlings under controlled conditions, they produced burning of the apple foliage in from 10 to 21 days. No description of characteristic symptoms of apple injury was given, however.

The literature on feeding habits of apple-inhabiting aphids also refers to curling of the leaves. The rosy apple aphid, *Anuraphis roseus*, is well known for its severe injury to apple trees by curling and twisting the leaves in clusters and often rendering fruits unmarketable (Baker and Turner (3), Matheson (17) and others). The green apple aphid, *Aphis pomi*, as reported by Matheson (17), is also able to contort the leaves, but the experiments conducted by Baker and Turner (2) indicate that this aphid causes very little leafcurling. The observations, as well as experiments, of several workers show that the apple grain aphid, *Rhopalosiphum prunifoliae*, seldom, if ever, causes any marked curling of the leaves.

Ackerman (1) differentiates the leafhopper curling of apple foliage from that of aphids. He states that the leafhopper curling begins "at the apex and extends toward the base of the leaves, the lower surface always being rolled in. This type of injury differs from aphid leaf-curl in that

aphids roll the leaves more tightly and curl them from the sides instead of from the tips."

Again, in discussing the types of injury by aphids, no reference is made to characteristic discoloration symptoms and marginal upward curling of the leaves.

EXPERIMENTAL EVIDENCE OF THE DISEASE

In order to determine the cause of this injury, several series of cage experiments were conducted at the Peninsular Branch Experiment Station, Sturgeon Bay, Wisconsin. The first preliminary series of experiments was started on July 10, 1924, in the orchard at the station grounds, and closed on August 11, 1924. The second series of experiments was conducted between May 20 and August 13, 1925. In all experiments each spur under observation was covered with a large parchment bag of a type commonly used in plant breeding, which allowed enough light for normal development of leaves and at the same time protected them from insects and other external contaminating factors.

On July 10, 1924, ten rapidly growing terminal spurs without evidence of symptoms, and apparently healthy, on Wealthy apple trees were selected. Only ten upper leaves were left on each spur, the lower leaves being stripped off the shoot. Twenty leaf-hoppers of different instars were placed on the foliage of five of these spurs and inclosed by the large parchment bags.

TABLE 1.—*Relation of feeding of Empoasca fabae to apple leaf injury, 1924*

No. of spur	Spurs with leafhoppers			Total	No. of spur	Spurs without leafhoppers			Total
	No. leaves showing injury					No. leaves showing injury			
	Severe	Slight	None			Severe	Slight	None	
1	6	3	1	10	1a	0	0	10	10
2	8	0	2	10	2a	0	2	8	10
3	3	4	3	10	3a	0	0	10	10
4	5	2	3	10	4a	0	0	10	10
5	5	4	1	10	5a	1	0	9	10

The other five spurs were bagged without insects to serve as controls. They were left undisturbed until August 11, 1924. When the spurs were examined, most of the leafhoppers were dead, but the injury was very characteristic and severe, being much more pronounced than in the open. This was probably due to a prolonged confinement of leafhoppers to the same foliage, which seldom happens in nature, where adult leafhoppers are swiftly moving from one leaf to another and from spur to spur. The control tips

remained healthy, having only one leaf out of fifty that showed the typical symptoms, while two leaves had only a slight trace of yellowing along the veins. This, however, was probably due to a feeding of leafhoppers on these leaves before the tips were caged. Table 1 summarizes the results.

Early in the summer of 1925 the above experiment was repeated, proper precautions being taken to protect the terminal shoots by caging them before the migration of this species of leafhoppers to apple foliage. The spurs were caged on May 20, when apple leaves just opened, and only a very few adult leafhoppers were present on the apple trees. During the second week of June the leafhopper population increased considerably. On June 11, ten protected terminal shoots were taken for the leafhopper feeding experiment. Five of them were colonized with ten adult leafhoppers each, using *Empoasca fabae*, and five shoots were kept protected as controls.

During the course of the experiment, the leaves were examined three times for symptoms. On June 18, most of the leaves showed a considerable downward curling and only a few showed a decided tip burning, with a slight yellowing just preceding the brown burnt areas. On July 3, when the spurs were examined for the second time, they were infested with both the adults and their nymphs of different ages. The spurs increased in length, and practically all of the younger leaves were badly curled downward, showing typical leafhopper feeding injury. Many leaves had their tips and apical margins brown from burning. Practically all of the older leaves and many younger ones displayed the characteristic discoloration along the midrib and lateral veinlets. The symptoms were invariably more progressive on the lower side of the veins than on the upper. Many leaves showed very severe injury. The third and the last examination of the shoots took place on July 14, when it was observed that many leaves were so badly damaged that they were not only discolored along the veins and turned up at the margins, but some of them were entirely dried out and fell off the spurs.

One shoot in this experiment was less affected than others, showing only the feeding injury and very little discoloration along the veins. This fact suggests the thought that probably not all of the leafhoppers of this species are able to produce the characteristic symptoms. It also points to a possible acquisition of virulence by leafhoppers during the feeding on wild hosts before reaching the apple trees. It may be that this tree was resistant to such an attack. This question is not clear, however, and requires further study.

The control shoots had all 51 leaves normal and healthy, without any symptoms of discoloration, curling or burning. Table 2 summarizes the results obtained in feeding of leafhoppers under controlled conditions.

TABLE 2.—*Relation of feeding of Empoasca fabae to apple leaf injury, 1925*

No. of spur	Number of leaves showing a characteristic injury					Total no. of leaves
	Very severe burning	Severe burning and discoloration	Strong discoloration along the veins	Slight discoloration	Only curling due to feeding	
1	4	2	2	1	0	9
2	0	0	0	2	5	7
3	7	3	2	0	0	12
4	5	4	1	0	0	10
5	6	3	1	0	1	11

This experiment, it is believed, definitely indicates that *Empoasca fabae* is responsible not only for curling the leaves due to feeding, but also for the characteristic discoloration along the veins and burning of the foliage, which resembles that of hopperburn of potatoes. Prolonged confinement of leafhoppers on the same foliage considerably intensifies the symptoms.

A few supplementary experiments were carried on in which only one or two leafhoppers were caged over one leaf on each tip, which in turn was also covered with a large bag for protection from other insects. The experiment showed that the downward curling of the young leaves is always associated with the feeding of leafhoppers. Typical discoloration symptoms, although present in the majority of the cases, were not, however, shown in all of them. This indicates the presence of some specific toxic substance or infectious material within the insects, the nature of which is not clearly understood. It was also found that it has a decided downward movement along the midrib and lateral veins, but is restricted to the leaves on which insects were feeding, other leaves of the same tips remaining free from the symptoms.

Similar feeding experiments were conducted with three common apple aphids, colonizing each species separately on leaves of rapidly growing apple tips. The most common aphid in apple orchards in the spring was found to be the apple-grain aphid, *Rhopalosiphum prunifoliae*. Five tips colonized with this species gave negative results so far as the symptoms in *pomi*, which curls the leaves but slightly, hardly tipping the apical ends downward. The rosy apple aphid, *Anuraphis roseus*, was less numerous, but it certainly produced very severe curling and sidewise twisting of the leaves with pronounced pocketing and deformations. Not in a single case, however, were there produced discoloration symptoms in any way comparable to those obtained in feeding of leafhoppers.

ARTIFICIAL PRODUCTION OF SYMPTOMS

Several attempts were made to produce symptoms by artificial inoculations, using inocula prepared by macerating the adults and advanced nymphal stages of *Empoasca fabae* in a small quantity of sterile distilled water in a test tube. The ordinary method of needle inoculation was used, having a small amount of cotton tied to the point of the needle to facilitate the introduction of a sufficient amount of inoculum into each of the needle pricks. Ten fine pricks, aiming to wound the midrib and lateral veinlets, were made on each leaf. Fifty-five leaves, which were protected from insects from May 20 to the end of the experiment, were thus inoculated on June 15, 1925. Three days later on June 18, the inoculated leaves were examined, but no symptoms were noticed with the exception of brown scars in the places of inoculation. On June 23 the second examination was made, and at this time 29 leaves showed progressive discoloration, variable in degree, but similar in all respects to symptoms obtained in the feeding experiments. When the treated tips were examined for the last time on July 3, all but seven of the leaves exhibited the symptoms in varying degrees from slight to severe. Fifty other leaves inoculated with sterile distilled water failed to show any symptoms more than a few dead cells around the needle pricks. This seems to indicate that the adults and advanced nymphal stages of insects contain some active principle which is capable of inducing the symptoms in the leaves.

Two series of inoculation experiments were performed, one between July 10 and August 11, 1924, and another between June 18 and July 14, 1925, by crushing the nymphs and adults on apple leaves, aiding the entrance of insect juice into the plant tissue by needle pricks. Five insects were used to each leaf. Thirty-one per cent of the leaves showed decided symptoms in different degrees. Evidently it is difficult to inoculate the leaves by this method, for the reason that small insects dry too rapidly after they are crushed on the leaves. Sufficient data was obtained, however, to indicate that insects carry some toxic substance or infectious agent to cause the symptoms.

An attempt was made to produce the symptoms by using the sap pressed out of leaves showing different stages of the disease. This work started on July 3 and was in progress until August 13, 1925. The ordinary method of needle inoculation was used, 10 pricks being made in each leaf. Fifty leaves were inoculated with the sap of apparently uninfected leaves as a check. At the close of the experiment all check leaves failed to show injury. Only 3 leaves out of 50 inoculated with the sap of diseased leaves showed decided discoloration. Four leaves showed a faint yellowing, which, however, was not very similar to the typical symptoms. In case of the

potato hopperburn, Eyer (12) obtained positive results in reinoculation made from the diseased tissues, while Fenton and Ressler (13) obtained only negative results, with some browning at the tips of potato leaflets. It is considered that data obtained in this experiment is not sufficiently convincing to warrant any definite conclusions. Yet it is quite possible that a virus from the sap of affected leaves may be transmitted to healthy ones, although giving a relatively very small percentage of infection on account of dilution of infective principle in leaf tissue and the crude artificial inoculation methods employed, as compared with those at the command of a sucking insect.

LEAFHOPPERS AND VIRUS DISEASES OF PLANTS

In connection with these experiments, it may be of interest to note that there are several very important virus diseases of economic plants attributed to feeding of leafhoppers. It will be sufficient to call to mind only a few well known instances in order to appreciate the importance of leafhoppers in transmission and dissemination of virus diseases. The curly-top of sugar beets is produced by punctures of the beet leafhopper, *Eutettix tenella* Baker, as is shown by the works of Ball, Severin, Carsner and others. The potato hopperburn is caused by the potato leafhopper *Empoasca fabae* (Harris) as demonstrated by Ball, Fenton, Eyer, etc.. The recent work by Kunkel definitely indicates that the aster leafhopper, *Cicadula sexnotata* (Fallen), is responsible for transmission of the aster yellows. There are a few other cases reported, and no doubt, in the course of time, many more will come to the attention of phytopathologists and entomologists.

In comparing the symptoms and the nature of better known cases, it may be said that there are at least two general types of virus diseases produced by leafhoppers. In case of the curly-top of sugar beets and the aster yellows, the infective principle is diffused and becomes systemic within the plant. The symptoms are usually more apparent in the new growth. In the case of the potato hopperburn, as well as in the symptoms described in this paper, the infective principle is localized in the foliage on which leafhoppers have had opportunity to feed.

It is believed that leafhoppers are equally as, or more important than, the aphids in dissemination of virus diseases, but on account of their agile habits are less suspected as an important factor in the spread of these diseases.

SUMMARY

1. The apple hopperburn symptoms are distinct from the ordinary feeding injury caused by leafhoppers.

2. The typical leafhopper feeding injury is characterized by downward curling of the leaves, which begins at the apex and extends toward the base of the leaves. In this curling the lower surface of the foliage is always rolled in, without a characteristic marginal discoloration along the lateral veins.

3. The apple hopperburn is characterized by marginal yellow discoloration of the leaves which moves progressively downward along the lateral veinlets and midrib. It is invariably more rapid in its development on the proximal side of the lateral veins than on the distal in reference to the petiole of the leaf. In cases of severe injury, the apical margins of the leaves turn brown and often curl upward.

4. Feeding experiments indicate that the potato leafhopper, *Empoasca fabae* (Harris), is not only able to produce the symptoms, but prolonged confinement of insects on apple leaves intensifies the symptoms.

5. These symptoms, observed on apple leaves, strongly resemble the hopperburn of potato, suggesting the existence of some toxic or specific infective principle within the insect.

6. This specific infective principle moves downward in vascular bundles, discoloring the veins, but is localized in the leaves subjected to leafhopper feeding.

7. The symptoms can be produced artificially by needle inoculations with inoculum prepared from macerated adults and advanced nymphal stages of the insect.

8. Crushing the nymphs and adults on apple leaves resulted in a smaller percentage of infection.

9. The sap of affected leaves gives only a very small number of infections, probably due to the crudeness of inoculation technique and to dilution of infective principle in the sap of the plant.

10. The definite association of certain species of leafhoppers with the symptoms; the progressive downward movement of symptoms in the vascular system; and artificial production of symptoms with inocula prepared from the insects and affected leaves indicate that the insect in question is a carrier of some specific infective principle, possibly a virus, in nature.

11. The leafhoppers may be looked upon as a major factor in dissemination and transmission of important virus diseases of plants.

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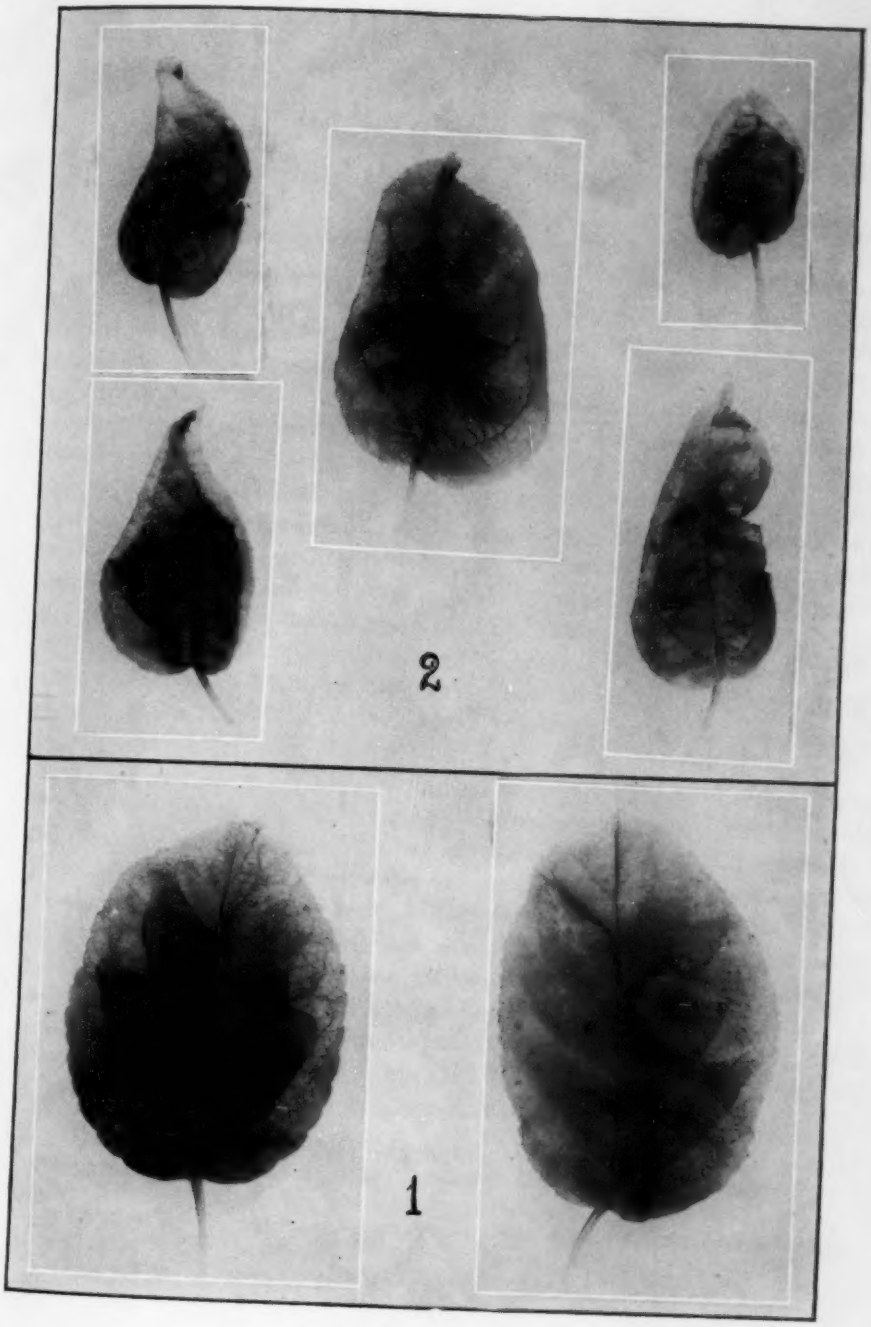
EXPLANATION OF PLATE XXIV

1. Two apple leaves showing a characteristic marginal discoloration, which gradually progresses downward along the midribs and lateral veins in a more or less triangular fashion. It is more rapid on the lower or proximal side of the veins than on the upper or distal side.

2. Several young apple leaves showing advanced stages of "hopperburn," which is characterized by upward curling of leaf margins.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STRAWBERRY "YELLOWS," A DEGENERATION DISEASE OF THE STRAWBERRY[1, 2]

A. G. PLAKIDAS

Strawberry plants in the Central California Coast District have suffered for a long time from an obscure disease. This disease, however, although more destructive in this section than in any other strawberry-growing district, is by no means limited to this region; it occurs in other parts of California, and also in the states of Oregon and Washington.

This trouble has been attributed by the growers to various causes, such as insect injury, too much water, soil conditions, variety degeneration, etc. The evidence at hand shows, however, that this is a new addition to the long list of plant troubles designated as "degeneration," "mosaic," or "virus" diseases; and, as far as the writer is aware, it is the first disease of this nature to be reported on the strawberry. Professor W. T. Horne[3] was the first investigator to recognize the fact that a specific disease was involved and gave a pathological description of it in 1922.

The most conspicuous symptoms of the disease are: (a) a characteristic curling or cupping of the leaves; (b) yellowing of the leaves around the margin and between the larger veins; (c) dwarfing of the leaves, both petioles and blades, and a general stunting of the growth of the entire plant; (d) premature reddening or autumnal color of the older outer leaves. (This is not a very definite symptom, because the leaves of healthy plants also turn reddish brown with age or when affected by frost. However, the leaves of diseased plants turn color earlier than those of healthy plants of the same age.) (e) Appearance of small red dots on the lobes of the margin of very young leaves. (These red dots appear on older leaves, whether healthy or diseased, but they are not present on the very young leaves of healthy vines, while they are always present on the young leaves of diseased ones.) (f) The disease is transmitted through the runners; *i.e.*, every daughter plant will be diseased. (g) The root system appears perfectly healthy during the early stages of the disease.

[1] The term "yellows" has been suggested to the writer by Prof. H. P. Barss, "because of characteristics reminding one somewhat of such virus diseases as aster yellows, peach yellows, etc."

[2] Plakidas, A. G. An obscure new disease of the strawberry in California. Phytopath. 15: 730. 1924. [Abstract].

[3] Rept. of the College of Agr. and Exp. Sta. Univ. of Calif. 122–123. 1921–22.

These symptoms are, of course, characteristic of typical cases. Some of the symptoms may be screened, or not conspicuously manifested under very favorable growing conditions, but again become apparent if the vigorous growth of the plant is checked. Plants may appear to recover at times, but again relapse. Complete recovery of an affected plant never has been noted. The disease is not transmitted through the seed. Seedlings from seed of diseased plants are healthy until they become inoculated.

The following have been considered as possible causes of the disease: the red spider (*Tetranychus telarius* Linn.), adverse chemical conditions of the soil, a soil organism, a virus.

Experiments have shown that no one of the first three is the cause of the disease. The detailed results of these experiments will be presented in a later publication. In this paper only the virus feature of the problem will be considered.

That "yellows" is a virus disease, and that insects act as vectors, is seen from the following circumstantial evidence:

(a) The disease is systemic; all daughter plants coming from diseased mother plants are diseased, and continue to produce diseased plants no matter on what soil they are allowed to grow.

(b) The infection spreads very rapidly and in the direction of the prevailing wind—just what we would expect if insects are the carriers of the infectious principle.

(c) The disease is not transmitted through seed: this is characteristic of diseases of this type.

The results of the following experiments give direct evidence that the disease is caused by a virus. A lot of 40 healthy young plants (variety Banner) in pots on steam sterilized greenhouse soil were divided into five sets of eight pots each and were treated as follows: set A was placed in an isolated spot, away from any other strawberry plants, to be used as check. The plants of set B were placed in another isolated spot and were lightly infested with red spider (*Tetranychus telarius* Linn.) taken from leaves of diseased plants. Similarly, the plants of set C were isolated and were infested each with 10 individual strawberry aphids (*Myzus fragaefolii*) taken from leaves of diseased plants. The pots of set D were placed in a patch of diseased plants and were left unprotected from insects. The diseased plants in the patch were infested with various insects—aphids, white flies, thrips, red spiders, etc. The plants of set E were placed in insect-proof cages in the vicinity of this same patch. This experiment was started the middle of July, 1925.

The results of these experiments are discussed below.

Set A (the control) remained healthy, and are still healthy at the present time. These plants became infested with insects—aphids, white flies, red spider, etc.—but apparently these insects did not come in contact with diseased plants. The plants of sets C and D (*i.e.*, those infested with "infected" aphids, and those left exposed to "infected" insects respectively) began to show "yellows" symptoms about one month after inoculation, and developed all the typical "yellows" symptoms after another month (Fig. 1).

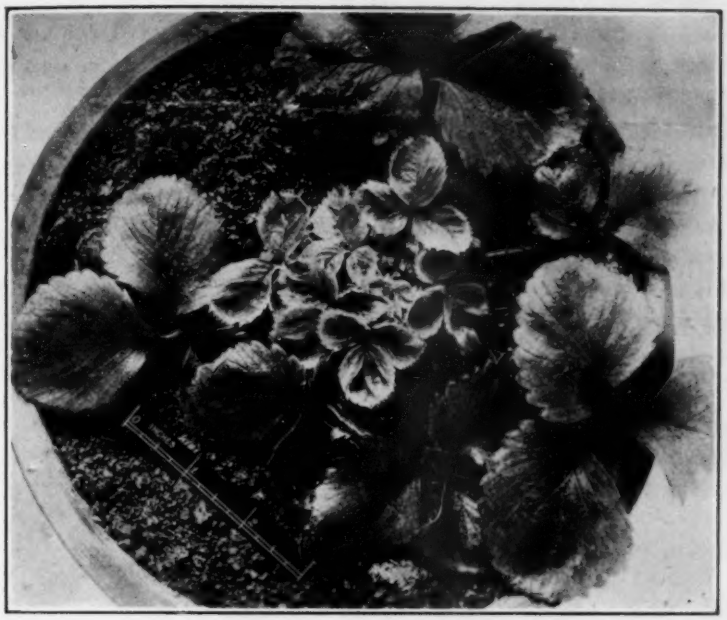

FIG. 1. Young Banner strawberry plant showing typical "yellows" symptoms.

Only 3 out of 8 plants of set B showed symptoms of the disease one month after inoculation; after another five weeks, all plants of this set were diseased. A doubt, however, remains as to whether the red spider inoculated all the plants in this set, or only the three that showed the symptoms first; for soon after the first three plants began to develop the "yellows" symptoms, the plants of this set were infested with strawberry aphids, and it is not improbable that these aphids inoculated the remaining five plants from the first three.

Six of the 8 plants which were placed in cages failed to grow (a severe treatment of nicotine sulphate-oil solution at the time of planting proved too strong for them) but the two that survived developed normally and are still healthy at present.

There is a possibility that this disease is transmissible by direct inoculation with juice of diseased plants, but the evidence at hand at present is inconclusive. Nine out of 11 apparently healthy plants inoculated by means of a hypodermic syringe with extracted juice from leaves of sick plants developed the "yellows," as against 4 out of 11 for each of two control sets—one injected with sterile water, and the other with juice from healthy plants. But, as the plants experimented with were not protected from insects, little, if any, weight can be placed on these results.

Effective insect-proof cages have been constructed, and further experiments are now in progress in order to learn more of the mode and agents of transmission of this disease.

Cytological examination of stained sections of roots, leaves, and petioles of diseased plants so far have failed to show the presence of any intracellular bodies or other abnormalities which are generally found associated with most virus diseases. Further study is being made in this direction, however.

This is a preliminary report of a general study of strawberry diseases carried on in this laboratory under the direction of Professor Ralph E. Smith.

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REVISED METHOD FOR THE QUANTITATIVE DETERMINATION OF SULFUR FUNGICIDES ON FOLIAGE

H. W. Fitch

In a previous article[1] the writer described a method for making a quantitative determination of sulfur fungicides on foliage. The following is a revision of that method and is much more accurate. For each 100 leaves, proceed as follows:

Pour 250 cc. of carbon tetrachloride into a large beaker. Immerse about 35 leaves in the tetrachloride and let stand, with occasional shaking, for 10 minutes. Then remove a few leaves at a time, pulling them out by the stems, and rinse them in a second beaker containing 250 cc. of carbon tetrachloride, carefully shaking off all droplets of solvent before discarding leaves.

Filter the carbon tetrachloride solution through a thin tuft of absorbent cotton in a funnel to remove coarse debris. Collect the filtrate in a 500 cc. flat bottomed flask and distill nearly to dryness on the hot plate through a condensor to recover the solvent. Filter more solution into the same flask, after cooling, until all the solution is filtered and distilled. Rinse the two beakers with Q. S. tetrachloride, passing rinsings through cotton wool, and distill as before.

Add 200 cc. distilled water to the residue in the flask, and then 5 grams sodium peroxide. Heat on the hot plate for 2 hours. Cool and add an additional 5 grams of peroxide. Heat an additional 2 hours. Cool and make decidedly acid to methyl red by addition of concentrated hydrochloric acid C.P. Heat about 1 hour on the hot plate and cool. Then place in the flask a small quantity of dry asbestos fiber, such as is used for Gooch crucibles, and shake well. The asbestos fiber will collect all flocculent waxes, etc., which are thrown out on acidifying.

Next prepare a Büchner funnel as follows:

Place in funnel two filter papers, wet, and sucked dry. Then form a fairly thick mat of asbestos fiber on the filter paper and suck dry. Next place a small watch glass on the asbestos mat, raising one edge by placing a short length of glass rod under it.

[1] Fitch, H. W. Quantitative determination of sulphur fungicides on foliage. Phytopath. **15**: 351–354. 1925.

The acid solution may now be filtered readily, pouring it on the watch glass in order not to disturb the asbestos mat. Wash well with three 75-cc. portions of distilled water.

Now neutralize the caustic soda until the solution is only faintly acid to methyl red, and determine barium sulfate in the usual way. When filtering the barium sulfate precipitate, test wash water with silver nitrate until free from chlorides.

Note: All re-agents, especially the sodium peroxide, must be free from sulfates.

For larger quantities of leaves, the same procedure is employed, increasing the quantities of re-agents used accordingly.

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PHYTOPATHOLOGICAL NOTES

The Sylloge Fungorum vol. 23.—The twenty-third volume of Saccardo's invaluable Sylloge Fungorum, issued by Dr. Alex. Trotter, treats of the Basidiomycetes and contains also, besides two pages of necrology and the announcement that volumes 24 and 25 are forthcoming, a brief tribute to Saccardo and a bibliography of his writings numbering 238 titles.

To the Hymnomycetes are given 583 pages; to the Gastromycetes 23 pages, to the Ustilaginaceae 27 pages, and to the Uredinales 317 pages.

Some conception of the records given may be gleaned from the fact that 82 numbers are given to *Uromyces*, 331 to *Puccinia*, 36 to *Ravenelia*, 43 to *Ustilago*, 108 to *Polyporus*, and 66 to *Clitocybe*. The mode of treatment is that of earlier volumes of the Sylloge.—F. L. Stevens.

Glomerella cingulata.—In a recent article (Mix, A. J., Anthracnose of European privet. Phytopath. **15**: 261–272. 1925), the name of the fungus causing privet anthracnose was given as *Glomerella cingulata* (Atk.) Spaulding and von Schrenk. This should have been: *Glomerella cingulata* (Stoneman) Spaulding and von Schrenk.—A. J. Mix.